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(54) INHIBITION OF THE ALTERNATIVE COMPLEMENT PATHWAY FOR TREATMENT OF TRAUMATIC BRAIN INJURY, SPINAL CORD INJURY AND RELATED CONDITIONS

HEMMUNG DES ALTERNATIVEN KOMPLEMENTWEGES ZUR BEHANDLUNG TRAUMATISCHER GEHIRNVERLETZUNGEN, RÜCKENMARKSVERLETZUNGEN UND VERWANDTER LEIDEN

INHIBITION DE LA VOIE DE COMPLÉMENT ALTERNATIVE POUR LE TRAITEMENT DE LÉSIONS TRAUMATIQUES DU CERVEAU, DE LÉSIONS DE LA MOELLE ÉPINIÈRE ET DE CONDITIONS APPARENTÉES

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Description

Field of the Invention

[0001] The present invention generally relates to methods of treating physiological damage resulting from traumatic brain injury, spinal cord injury, or related conditions, by selectively inhibiting the alternative complement pathway, and in a particular embodiment, by inhibiting factor B.

Background of the Invention

[0002] Complement activation occurs primarily by three pathways: the so-called classical pathway, the lectin pathway and the alternative pathway. The key proteins involved in the activation of the alternative pathway are factor B (fB) and factor D (fD). These proteins work in concert to initiate and/or to amplify the activation of C3, which then leads to the initiation of a number of inflammatory events. A third protein, properdin, stabilizes the complex of C3 and factor B but is not absolutely required for the alternative pathway to function. Factor B also helps solubilize immune complexes, has been reported to act as a B cell growth factor and can activate monocytes (Takahashi, 1980; Hall, 1982; Peters, 1988). Factor B-deficient mice (*fB*-/-mice) have been generated and IgG1 antibody response to T-cell dependent antigens and sensitivity to endotoxic shock appear normal in these mice (Matsumoto, 1997).

[0003] The alternative complement pathway is usually initiated by bacteria, parasites, viruses or fungi, although IgA Abs and certain Ig L chains have also been reported to activate this pathway. Alternative pathway activation is initiated when circulating factor B binds to activated C3 (either C3b or C3H2O). This complex is then cleaved by circulating factor D to yield an enzymatically active fragment, C3bBb. C3bBb cleaves C3 generating C3b, which drives inflammation and also further amplifiers the activation process, generating a positive feedback loop. Both components (factor B and factor D) are required to enable activation of the alternative pathway.

[0004] Recent studies have shown that the alternative pathway of complement plays an important role in the pathogenesis of several animal models of disease. For example, complement activation within the kidney after ischemia reperfusion injury (I/R) is mediated almost exclusively by the alternative pathway (Thurman et al., 2003, J Immunol 170:1517-1523), and the alternative pathway plays a critical role in the development of inflammatory arthritis. Perhaps most surprisingly, mice deficient in the alternative pathway have been demonstrated to be protected from nephritis in the MRL/lpr model of lupus nephritis (Watanabe et al., 2000, J Immunol 164:786-794) and from anti-phospholipid mediated fetal loss (Girardi et al., 2003, J Clin Invest 112:1644-1654), models that would traditionally have been assumed to be mediated by the classical complement pathway. In addition, Nataf et al. has shown that, in an experimental autoimmune encephalomyelitis (EAE) model, in both C3(-/-) and factor B(-/-) mice, there was little infiltration of the parenchyma by macrophages and T cells and, as compared with their wild-type littermates, the central nervous systems (CNS) of both C3(-/-) and factor B(-/-) mice induced for EAE are protected from demyelination (Nataf et al., 2000, J. Immunol.165:5867-5873). Subsequent studies of autoimmune pathology in C4 (-/-) mice in the EAE model showed that deletion of the C4 gene does not significantly change either the time of onset or the severity and tempo of myelin oligodendrocyte-induced EAE compared with controls with a fully intact complement system, indicating that the contribution of murine complement to the pathogenesis of demyelinating disease is realized via the alternative pathway (Boos et al., 2005, Glia 49:158-160).

[0005] Traumatic brain injury (also referred to herein as TBI) is a condition with very deleterious effects on an individual's health that currently has no effective treatment. Complement activation has been shown to be involved in the development of brain damage following TBI (Bellander et al., 2001, J. Neurotrauma 18:1295-1311; Kaczorowski et al., 1995, J. Cereb. Blood Flow Metab. 15:860-864; Keeling et al., 2000, J. Neuroimmunol. 105:20-30; Schmidt et al., 2004, Eur. J. Trauma 30:135-149; Nataf et al., 1999, Trends Neurosci 22:397-402; Stahel et al., 1998, Brain Res. Rev. 27:243-256; Stahel et al., 2001, J. Neurotrauma 18:773-781; Van Beek et al., 2003, Ann NY Acad Sci 992:56-71; Rancan et al., 2003, J. Cereb. Blood Flow & Metab. 23:1070-1074). However, these studies have focused on the effects of the complement cascade at a point where all three pathways that activate complement converge, such as at C3 (see, for example, Rancan et al., 2003, *ibid*.). Therefore, prior to the present invention, there have been no reports showing whether one of the complement pathways is preferentially or exclusively activated as a result of TBI, or is required to develop TBI.

[0006] The immediate goal in the management of head-injured patients is the prevention of secondary brain damage by rapid correction of hypotension, hypoxemia, hypercarbia and hypoglycemia. The main priority in the early management of head trauma patients is the maintenance of an adequate cerebral perfusion pressure (CPP), which should be above 70-80 mmHg. Different therapeutic approaches are aimed at lowering the intracranial pressure (ICP) in order to keep an adequate CPP. Among the therapeutic modalities are: the reduction of mass lesions by surgical evacuation of intracranial hematomas, the reduction of brain swelling with osmotic drugs (*e.g.*, mannitol), and the therapeutic drainage of cerebrospinal fluid (CSF) through intraventricular catheters. Patients with severe TBI are transferred to intensive care unit (ICU) at the earliest timepoint and treated according to standardized protocols. Goals of ICU therapy include:

achievement and maintenance of adequate gas exchange and circulatory stability, prevention of hypoxemia and hypercarbia, repeated, scheduled computerized tomography (CT) scans for detection of delayed secondary intracranial pathology, profound sedation and analgesia to avoid stress and pain, achievement and maintenance of optimal CPP (> 70 mmHg) and cerebral oxygen balance, avoidance of hyperthermia (< 38°C), prevention of hyperglycemia and hyponatremia, no routinely performed head elevation, prevention of stress ulcers and maintenance of gut mucosal integrity, and prophylaxis for complicating factors (*e.g.* pneumonia or meningitis). In the event of elevated ICP (> 15 mmHg, > 5 minutes), patients can be treated by (1) deepening of sedation, analgesia, muscle relaxation; (2) CSF drainage through ventricular catheters; (3) moderate hyperventilation (under certain circumstances); (4) osmotherapy; (5) moderate hypothermia (± 34°C); and (6) barbiturate coma.

[0007] Spinal Cord Injury (also referred to herein as SCI) is also a condition of the central nervous system with very deleterious effects on an individual's health that currently has no effective treatment. Complement activation has been shown to be involved in the development of damage following SCI (Anderson et al., 2004, J Neurotrauma 21(12):1831-46; Reynolds et al., 2004, Ann N Y Acad Sci. 1035:165-78; Rebhun et al., 1991, Ann Allergy 66(4):335-8). However, as with TBI, these studies have focused on the effects of the complement cascade at a point where all three pathways that activate complement converge, or have suggested a role for all complement pathways subsequent to SCI. Therefore, prior to the present invention, there have been no reports showing whether one of the complement pathways is preferentially or exclusively activated as a result of SCI, or is required to develop SCI.

[0008] SCI is generally defined as damage to the spinal cord that results in a loss of function, such as mobility or feeling. Frequent causes of damage are trauma (*e.g.*, by car accident, gunshot, falls, etc.) or disease (polio, spina bifida, Friedreich's Ataxia, etc.). The spinal cord does not have to be severed in order for a loss of functioning to occur. In fact, in most individuals with SCI, the spinal cord is intact, but the damage to it results in loss of function. Besides a loss of sensation or motor function, individuals with SCI may also experience dysfunction of the bowel and bladder, sexual and fertility dysfunction, inability to regulate blood pressure effectively, reduced control of body temperature, inability to sweat below the level of injury, and chronic pain. Very high injuries (C-1, C-2) can result in a loss of many involuntary functions including the ability to breathe, necessitating breathing aids such as mechanical ventilators or diaphragmatic pacemakers.

[0009] Currently there is no cure for SCI. The immediate goal in the management of SCI patients is focused on decreasing damage as soon as possible after the injury occurs. Steroid drugs such as methylprednisolone reduce swelling, which is a common cause of secondary damage at the time of injury. There are several types of treatment in the short term for a spinal cord injury. First, the spine in the area of the injured spinal cord is immobilized to prevent further injury to the cord (*e.g.*, using halos, casts, braces and straps). To reduce swelling in the spinal cord caused by injury, steroid medication is usually given during the first 24 hours following injury, although the more typical approach is to give steroid medication to those patients with neurological deficits and a time window of initiation of therapy within less than 8 hours after trauma (Bracken, 2001, Spine 26(24S):S47-S54). Other medical treatment is often necessary, depending on complications that may develop. Because traumatic injury to the spinal cord usually involves an injury to the bones and ligaments of the spine, surgery may be performed. The aim of some surgeries is to remove bone (decompression) that is pressing on or into the spinal cord, or to stabilize or realign the spine in the area of the spinal cord injury when the vertebrae or ligaments have been damaged. Metal rods or cages and screws may be attached to normal vertebrae to prevent movement of fractured vertebrae and the vertebrae may be "fused" together using bone graft or the same reason. Stretching of the spine using weights and pulleys (called traction) may also help with alignment of the spine.

[0010] Despite the protocols for treatment of patients with TBI, potential complications from TBI therapy can include: cerebral vasospasms or cardiovascular depression, hepatotoxicity, immunosuppression, and increased incidence of pulmonary infections. In addition, although treatments for SCI may provide modest reductions in physiological damage, many protocols are primarily useful to help reduce the likelihood of further damage and to stabilize the patient. No single protocol has been proven to be entirely satisfactory for inhibiting the development of the physiological damage resulting from TBI or SCI. Therefore, there is a continuing need in the art for therapeutic processes and reagents having less toxicity and more specificity for the underlying cause of damage resulting from TBI and SCI.

Summary of the Invention

[0011] The present invention relates to an antibody or antigen binding fragment thereof for use in the reduction or prevention of at least one symptom of physiological damage resulting from traumatic brain injury (TBI) in an animal or enhance recovery from TBI or SCI in the animal, wherein the antibody or antigen-binding fragment thereof selectively binds to and inhibits the activity of factor B, factor D, or properdin, as claimed hereinafter. Preferred embodiments of the invention are set forth in the dependent claims.

[0012] In one aspect, the antibody or antigen-binding fragment thereof selectively binds to factor B within the third short consensus repeat (SCR) domain, wherein the antibody prevents formation of a C3bBb complex. In one aspect, the antibody or antigen-binding fragment thereof binds to factor B and prevents or inhibits cleavage of factor B by factor

D. In one aspect, the antibody or antigen binding fragment bind to the third short consensus repeat (SCR) domain of human factor B. In another aspect, the antibody or antigen binding fragment thereof selectively binds to an epitope in the third SCR domain of factor B selected from: (a) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from about position Tyr139 to about position Ser185, or equivalent positions thereto in a non-human factor B sequence; (b) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from about position Tyr139 to about position Ser141, or equivalent positions thereto in a non-human factor B sequence; (c) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from about position Glug182 to about position Ser185, or equivalent positions thereto in a non-human factor B sequence; and/or (d) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising any one or more of the following positions or their equivalent positions in a non-human factor B sequence: Tyr139, Cys 140, Ser141, Glu182, Gly184, or Ser185. In one aspect, the antibody or antigen binding fragment thereof selectively binds to an epitope in the third SCR domain of factor B (SEQ ID NO:2) comprising one or more of the following amino acid positions or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192. In another aspect, the antibody or antigen binding fragment thereof selectively binds to an epitope in the third SCR domain of factor B (SEQ ID NO:2) comprising or consisting of the following amino acid positions or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192. In yet another aspect, the antibody or antigen binding fragment thereof selectively binds to a non-linear epitope within the three-dimensional structure of a portion of the third SCR domain of factor B, wherein the portion is defined by at least amino acid positions Ala137-Ser192 of SEQ ID NO:2. The antibody or antigen binding fragment thereof may selectively bind to factor B from multiple mammalian species and prevents formation of a C3bBb complex. In one aspect, the antibody or antigen binding fragment thereof selectively binds to factor B from human and an animal selected from the group consisting of non-human primate, mouse, rat, pig, horse and rabbit. For any of the antibodies described above, the antibody can include, but is not limited to, an antibody of a non-complement activating isotype or subclass, a monoclonal antibody, a humanized antibody, a bispecific antibody, and/or a monovalent antibody. In one aspect, the antigen binding fragment is an Fab fragment. In one preferred aspect of the invention, the antibody is the monoclonal antibody 1379 (produced by ATCC Deposit No. PTA-6230), or an antigen-binding fragment thereof.

[0013] In the uses related to TBI, in a preferred embodiment, the agent is administered intravenously or to the brain of the animal. In the uses related to SCI, in a preferred embodiment, the agent is administered intravenously or to the spinal cord or epidural space of the spinal cord of the animal. The agent is preferably administered to the animal in an amount effective to measurably reduce at least one symptom of physiological damage resulting from TBI or SCI in the animal as compared to in the absence of administration of the agent. With respect to TBI, in one aspect, the agent is administered in an amount effective to maintain a cerebral perfusion pressure (CPP) of above 70-80 mmHg, or in an amount effective to lower intracranial pressure (ICP). With respect to SCI, in one aspect, the agent is administered in an amount effective to reduce swelling in the spinal cord. In one aspect, the agent is administered in a pharmaceutically acceptable carrier, including, but not limited to, a compound or composition that is capable of crossing the blood-brain barrier and/or an injectable excipient.

[0014] In one aspect of any of the uses described above related to TBI, there is a further step of administering to the animal another compound for treating a symptom of TBI selected from the group consisting of: a physical impairment, a cognitive impairment, and a psychosocial-behavioral-emotional impairment. Such a compound can include, but is not limited to, an osmotic drug, a sedative, an analgesic, a muscle relaxant, and/or a barbituate.

[0015] In one aspect of any of the uses described above related to SCI, there is a further step of administering a steroid to the animal.

[0016] In any of the above-described uses, the animal is preferably a mammal, including, but not limited to, a human.

[0017] Another embodiment of the present invention relates to a composition for use in the reduction or prevention of a symptom of traumatic brain injury (TBI) or spinal cord injury (SCI) in an animal or to enhance recovery from TBI or SCI in an animal wherein the composition comprises: (a) a first agent selected from the group consisting of an isolated antibody or an antigen-binding fragment thereof that selectively binds to and inhibits the activity of factor B, factor D or properdin; and (b) a second agent for use in the treatment of a symptom of traumatic brain injury (TBI) or SCI. The second agent may be a compound for treating a symptom of TBI selected from: a physical impairment, a cognitive impairment, and/or a psychosocial-behavioral-emotional impairment. The second agent may be selected from the group consisting of: an osmotic drug, a sedative, an analgesic, a muscle relaxant, and a barbituate.

(a) a first agent selected from: an isolated antibody, an antigen-binding fragment thereof, and/or an antigen-binding polypeptide, wherein the first agent selectively inhibits the expression or biological activity of a protein in the alternative complement pathway; and (b) the second agent may be a compound for treating a symptom of spinal cord injury (SCI). The second agent may be a steroid.

Brief Description of the Figures

[0018]

Fig. 1 is a schematic diagram showing the construction of a factor B-Ig fusion protein.
Fig. 2A is a line graph showing that anti-factor B completely inhibited the alternative complement pathway in a zymosan assay when 3 μg were added to a reaction containing 10 μl of serum.
Fig. 2B is a line graph showing that anti-factor B completely inhibited the alternative complement pathway in a rabbit erythrocyte lysis assay when 6 μg of antibody were added to 10 μl of human serum.
Fig. 3 is a line graph showing that administration of anti-factor B to mice inhibits the alternative complement pathway.
Fig. 4 is a schematic drawing showing a model of the epitope mapping for mAb1379 on the human factor B surface.
Fig. 5 is a schematic drawing showing a modeled complex of mAB1379 (one Fab fragment) binding to factor B, with the antigen binding sides of the Fab having been modeled to cover the entire mapped epitope region.
Fig. 6 is a line graph showing that Crry-Ig inhibits neurological impairment after TBI.
Fig. 7 is a line graph showing that Crry-Ig inhibits weight loss after TBI.
Fig. 8 is a bar graph showing that administration of anti-Factor B (mAb 1379) reduces brain damage associated with TBI.
Fig. 9 is a line graph showing that administration of anti-Factor B (mAb 1379) improves recovery from spinal cord injury.
Fig. 10 is a graph showing that elevated C5a levels in serum of brain-injured C57BL/6 (fB+/+) mice are significantly attenuated in factor B gene-deficient (fB-/-) mice lacking a functional alternative complement pathway.
Fig. 11 is a digital image of a Western blot showing the upregulation of the anti-apoptotic mediator Bcl-2 in serum and brains of fB-/- mice after traumatic brain injury (TBI), as determined by Western blot analysis.
Fig. 12 is a digital image showing attenuated neuronal cell death in the injured hemisphere of factor B gene-deficient mice 4 hours after closed head injury.
Fig. 13 is a digital image showing attenuated neuronal cell death in the injured hemisphere of factor B gene-deficient mice 24 hours after closed head injury.
Fig. 14 is a digital image showing attenuated neuronal cell death in the injured hemisphere of factor B gene-deficient mice 7 days after closed head injury.

Detailed Description of the Invention

[0019] The present invention generally relates to the inventors' discovery that activation of the complement cascade through the alternative pathway is necessary for the induction of physiological damage due to traumatic brain injury (TBI), and that inhibition of the alternative complement pathway is sufficient to reduce damage (or enhance recovery) resulting from TBI or spinal cord injury (SCI). More particularly, the present inventors disclose herein the discovery that inhibition of the alternative pathway inhibits physiological damage (*e.g.*, brain damage) in an experimental model of TBI, and also inhibits damage (measured by enhanced recovery) in an experimental model of SCI. Accordingly, the present invention relates to compounds, compositions and the use of such compounds or compositions in methods for the prevention and/or treatment of TBI, SCI or other neuronal or brain damage, through the selective inhibition of the alternative complement pathway.

[0020] First, the present inventors have shown for the first time a major role of the alternative pathway of complement activation in contributing to the overall extent of posttraumatic complement activation and to secondary neuronal cell death after brain injury. Furthermore, the inventors have demonstrated that specific inhibition of the alternative complement pathway by inhibition of factor B, in addition to general inhibition of the complement pathway through inhibition of C3 using a C3 complement convertase inhibitor, Crry-Ig, both inhibit damage associated with TBI. This is believed to be the first disclosure of the ability to inhibit physiological damage and effects associated with TBI by specifically and selectively inhibiting the alternative complement system.

[0021] Second, the present inventors have shown that inhibition of the alternative complement pathway through the inhibition of factor B inhibits damage associated with SCI. This is believed to be the first disclosure of the ability to inhibit physiological damage and effects associated with SCI by specifically and selectively inhibiting the alternative complement system.

[0022] The identification of factor B and the other proteins in the alternative complement pathway (e.g., factor D or properdin) as specific therapeutic targets provides a rational strategy as well as lead compounds that can be used to inhibit physiological damage or effects resulting from TBI or SCI via selective inhibition of the alternative complement pathway.

[0023] Several inhibitors have already been developed to inhibit the complement system at various stages of activation (Holers, V.M. 2003, Clin Immunol 107:140-151), although specific inhibitors of the alternative pathway have not been

widely reported. Specific inhibition of the alternative pathway has several advantages compared with existing inhibitors of the complement cascade. First, because the present inventors have discovered that physiological damage due to TBI or SCI is primarily mediated by the alternative pathway of complement activation, a specific inhibitor of this pathway will be equally effective as a pan-complement inhibitor, yet should have fewer immunosuppressive side-effects. Furthermore, *C4* -/- mice (mice lacking the C4 complement component that is generic to the classical, alternative and lectin complement pathways), but not *fB* -/- (factor B deficient) mice, appear more susceptible to systemic experimental bacterial infection, which suggests that by leaving the classical pathway intact, an inhibitor of the alternative pathway poses less risk for serious infection. Although only one human patient with congenital deficiency of factor B has been reported (Densen et al., 1996, Mol Immunol 33:68 (Abstract 270)), studies of gene targeted factor B deficient mice (*fB*-/-) have not yet demonstrated an immune-modulating effect for this factor (Densen et al., *supra*; Matsumoto et al., 1997, Proc Natl Acad Sci U S A 94:8720-8725). Patients with congenital deficiencies of classical pathway components, in contrast, appear to have an increased risk of infection (most commonly *Staphylococcus* and *Streptococcus*). Inhibition of classical pathway components or C3 (common to all of the complement pathways) might also be associated with autoimmunity (Figueroa and Densen, 1991, Clin Microbiol Rev 4:359-395), perhaps explaining why factor B deficiency protects MRL/lpr mice from developing glomerulonephritis, but C3 deficiency does not (Watanabe et al, *supra*). Selective inhibition of the alternative pathway prevents generation of C3-derived ligands for the C3a receptor as well as for complement receptors 1-4 and C5a. The effects of blocking of the alternative pathway may in fact be more direct, due to as yet poorly characterized receptors for the Ba or Bb activation products of factor B that are generated during the activation process. Thus, inhibition of the alternative pathway is expected to be better tolerated and more effective than classical pathway complement inhibition.

[0024] Given the great potential therapeutic benefit of an inhibitor specific for the alternative complement pathway for use in the methods of the present invention for treatment of TBI and SCI and related conditions, the present inventors have developed several inhibitory monoclonal antibodies directed against factor B and have tested one of them in an experimental model of TBI and also in an experimental model of SCI. These antibodies are described in detail in U.S. Patent Application Publication No. 2005-0260198-A1, published November 24, 2005 and in PCT Publication No. WO 2005/077417, published August 25, 2005.

[0025] Briefly, to produce the antibodies, gene targeted factor B-deficient mice (*fB*-/-) were injected with a fusion protein comprised of the second and third short consensus repeat (SCR) domains of factor B linked to the hinge, CH2, and CH3 domains of a mouse IgG1 isotype (see Fig. 1). These SCR domains were chosen because they are part of the deleted segment of the factor B gene in the *fB*-/- mice. Mice were screened for an immune response to factor B (*e.g.*, using ELISA), and spleen cells from one of the injected mice were fused to myeloma cells. One of the resulting hybridomas, named 1379, produces an IgG_1 antibody that inhibits alternative complement pathway activation *in vitro* (Figs. 2A and 2B) and *in vivo* (Fig. 3). Specifically, this antibody was tested in two *in vitro* assays of alternative pathway activity (Figs. 2A and 2B), and showed that the antibody can completely inhibit the lysis of erythrocytes by human serum, thus confirming the ability of this reagent to completely block alternative complement pathway activation. When mice were tested for inhibition of the alternative pathway at various times after a single injection of the inhibitory antibody, 1 mg of antibody led to full inhibition within one hour when injected IV and within two hours when injected IP (Fig. 3). Mice receiving a one mg injection IP retained full inhibition of the alternative pathway at 24 hours and those receiving a two mg injection retained full inhibition up to 48 hours after the injection. The inventors have also injected 2 mg of the 1379 antibody repetitively i.p. every other day for 14 days and have shown that the complete inhibition of the alternative complement pathway was maintained for at least 48 hours after the last injection. Moreover, Fab fragments made from this antibody also resulted in complete inhibition of the alternative pathway in approximately equimolar levels as with the intact 1379 antibody.

[0026] The 1379 antibody inhibits alternative pathway activation in serum from animals including, mice, rats, humans, baboons, rhesus monkeys, cyno monkeys, pigs, rabbits, and horses (Table 1).

Table 1

Species in which the alternative pathway is fully inhibited by mAb 1379
• Mouse
• Human
• Rat
• Baboon
• Rhesus
• Pig
• Cyno Monkey
• Horse

(continued)

Species in which the alternative pathway is not inhibited by mAb 1379
• Dog
• Guinea Pig

[0027] A panel of anti-factor B antibodies produced by the inventors is shown in Table 2. As discussed above, the inventors have shown that the mAb 1379 both binds and inhibits mouse and human factor B. In contrast, the mAb designated 624 can bind both mouse and human factor B, but does not inhibit the human alternative pathway. As revealed in a competition assay, antibodies 624, 691, and 1231 do not block binding by 1379. These antibodies must therefore bind the protein at a different site, explaining why they bind factor B without inhibiting its function *in vitro*. However, antibodies 395, 1322 and 1060 are competitive inhibitors of 1379.

Table 2

Clone	Isotype	Binds mouse fB	Binds human fB	Inhibits mouse alternative pathway (zymosan assay)	Inhibits human alternative pathway (rabbit erythrocyte lysis assay)	Competes with 1379 for human fB binding
1379	IgG1 κ	+++	+++	+++	+++	+++
395	IgG1 κ	+++	++	++	+++	+++
1322	IgG2b κ	+++	+++	+	++	+++
624	IgG1 κ	+++	+++	+	-	-
691	IgG1 κ	+++	+++	+	-	-
1060	IgG2b κ	+++	+++	+	++	++
1231	IgG1	+++	+++	+	-	-
E1128		-	+++	-	0	NA

[0028] Epitope mapping was used to demonstrate that this antibody binds to factor B within the third short consensus repeat (SCR) domain, and the antibody prevented formation of the C3bBb complex. In addition, experiments to map the epitope for the mAb1379 antibody indicated that the epitope or antibody binding site on factor B was not linear. Experiments demonstrated that the introduction of certain alanine substitutions into SCRs 2 and 3 of human factor B, but not SCR1, resulted in the loss or substantial loss of binding of the 1379 antibody to factor B, which included mutants that substituted: 139-Tyr-140-Cys-141-Ser with His-Cys-Pro (the positions being relevant to the mature human factor B represented by SEQ ID NO:2); and 182-Glu-183-Gly-184-Gly-185-Ser with Gly-Asn-Gly-Val.

[0029] The predicted conserved binding surface or epitope of the human factor B that is recognized by mAb1379 was modeled. Briefly, the tertiary structure of human factor B was built based on the resolved three-dimensional structure of CR2-SCR1-2 (Protein Data Bank (PDB) id 1GHQ). Fig. 4 shows the model of the factor B structure with the amino acid positions corresponding to the mAb1379 epitope (relative to SEQ ID NO:2) indicated. The residues that are believed to form the conformational epitope for the mAb1379 antibody are: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192, although the epitope may contain only a few, substantially all, or more residues than is depicted in Fig. 4. Fig. 5 is a schematic drawing showing a modeled complex of mAB1379 (one Fab fragment) binding to factor B, with the antigen binding sides of the Fab having been modeled to cover the entire mapped epitope region as defined above in Fig. 4.

[0030] The antibodies that have been produced by the present inventors, described in more detail in U.S. Patent Application Publication No. 2005-0260198-A1 and in PCT Publication No. WO 2005/077417, *supra,* recognize a site on factor B that is shared among humans and many other animal species in which pre-clinical proof-of-principle experiments are performed, thus allowing discoveries in models of human disease to be readily translated into human therapies. These antibodies are believed to be the first antibodies against factor B that exhibit the broad species inhibition of the protein. Therefore, a unique site has also been identified on factor B against which new inhibitory reagents can be developed.

[0031] In the present invention, the inventors have discovered and report for the first time herein that inhibition of the alternative pathway inhibits physiological damage in traumatic brain injury (TBI), and also inhibits physiological damage in spinal cord injuray (SCI) and this information can now be used to design, isolate and/or identify novel therapeutic

reagents for the treatment of TBI and SCI. Moreover, the antibodies previously produced and described by the inventors are excellent agents for use in the methods of the present invention.

[0032] One embodiment of the present invention relates to a method to reduce or prevent at least one symptom or condition (disability, impairment, physiological damage) resulting from (associated with) traumatic brain injury (TBI) in an animal to or enhance (increase) the recovery from damage caused by TBI, comprising selectively inhibiting the alternative complement pathway in an animal that has experienced TBI. Another embodiment of the invention relates to a method to reduce or prevent at least one symptom or condition (disability, impairment, physiological damage) resulting from (associated with) spinal cord injury (SCI) in an animal or enhance (increase) the recovery from damage caused by SCI, comprising selectively inhibiting the alternative complement pathway in an animal that has experienced SCI. In one preferred embodiment, the method includes administering to the animal an agent that inhibits the alternate complement pathway, and particularly, an agent that inhibits factor B. In one particularly preferred embodiment, the agent is an anti-factor B antibody or an antigen binding fragment thereof.

[0033] Accordingly, the methods of the present invention include a step of selectively inhibiting the alternative complement pathway in an animal that has, or is at risk of developing, physiological damage due to TBI or SCI, respectively. According to the present invention, to inhibit the alternative complement pathway in an animal refers to inhibiting the expression and/or the biological activity of at least one protein or nucleic acid molecule encoding such protein that is part of the alternative complement pathway. Such proteins include, but are not limited to, factor B, factor D or properdin. To "selectively" inhibit the alternative complement pathway means that the method of the present invention preferentially or exclusively inhibits the alternative complement pathway, but does not inhibit or at least does not substantially inhibit other pathways for complement activation, including the classical complement pathway or the lectin pathway. For example, the novel factor B antibodies and antigen binding fragments thereof of the present invention are one example of a reagent that selectively inhibits the alternative complement pathway. To "selectively" inhibit a specific protein means that the method of the present invention preferentially or exclusively inhibits the expression and/or a biological activity of the specific protein, but does not inhibit or at least does not substantially inhibit the expression and/or a biological activity of other proteins (unless such biological activity is one that is shared, such as a downstream event, with the specific protein).

[0034] According to the present invention, traumatic brain injury (TBI) is defined as any injury, wound, or damage caused by any type of trauma to the head, such as impact to the head or shaking. More specifically, TBI is an acquired injury to the brain caused by an external physical force, resulting in total or partial functional disability or psychosocial impairment, or both. The term applies to open and closed head injuries resulting in impairments in one or more areas, such as cognition; language; memory; attention; reasoning; abstract thinking; judgment; problem-solving; sensory, perceptual, and motor abilities; psychosocial behavior; physical functions; information processing; and speech. The term typically does not apply to brain injuries that are congenital or degenerative, or brain injuries induced by birth trauma, although the latter type of trauma may also be treated using the method of the invention. TBI can result in a variety of physiological and psychological symptoms, conditions or impairments, including physical impairments (e.g., speech, vision, hearing and other sensory impairment; headaches; lack of fine motor coordination; spasticity of muscles; paresis or paralysis of one or both sides and seizure disorders; balance impairments; and other gait impairments), cognitive impairments (e.g., short- and long-term memory deficits, impaired concentration, slowness of thinking and limited attention span, as well as impairments of perception, communication, reading and writing skills, planning, sequencing, and judgment), and psychosocial-behavioral-emotional impairments (e.g., fatigue, mood swings, denial, self-centeredness, anxiety, depression, lowered self-esteem, sexual dysfunction, restlessness, lack of motivation, inability to self-monitor, difficulty with emotional control, inability to cope, agitation, excessive laughing or crying, and difficulty relating to others). A detailed discussion of the diagnosis of TBI has been presented above.

[0035] Methods for diagnosing TBI are well-established in the art. Typically, TBI is diagnosed by the history of trauma, the clinical status and imaging studies, such as x-rays and computerized tomography (CT) scan. Of particular importance is the use of the post-resuscitation Glasgow Coma Scale (GCS) score (Teasdale and Jennett, 1974, Lancet 2 (7872):81-84), since this parameter represents an important predictor of outcome. When assessing the GCS, the *best* response is used to calculate the score. Patients with *mild* head injury (GCS 14 or 15) represent about 80% of all head trauma patients admitted to the emergency department. Moderate head injury corresponds to a GCS score between 9 and 13 and is associated with an increased risk for intracranial pathology compared to patients with *mild* head injury. A GCS score of 8 points or less corresponds to a comatose patient, as defined by the inability to open the eyes, to obey commands and to respond verbally. Thus, severe head injury is defined as a GCS score of 3 to 8. When evaluating the patient, in addition to the GCS and assessment of the level of consciousness, a neurologic exam typically includes the assessment of pupillary size and reactivity and a brief evaluation of peripheral motor function. The clinical exam furthermore includes the inspection of the scalp for lacerations, palpation of the skull for impression fractures and the search for indirect signs of basilar skull fractures, including periorbital ecchymosis ("racoon eyes"), retroauricular ecchymosis ("Battle's sign"), rhinorrhoea / otorrhoea due to CSF leakage, and VIIth nerve palsy. Under certain circumstances, a CT scan is given. Other causes of coma or altered state of consciousness may be investigated in the analysis, such as by

screening for: drugs, metabolic dysfunction, internal or external bleeding sources, preexisting non-traumatic brain damage (e.g., ischemic or hemorrhagic brain injury), epilepsy, basilar artery thrombosis, bacterial meningitis, brain abscess or tumor. Morphological classification of closed head injury is based on findings in the CT scan according to the guidelines of Marshall and colleagues (Marshall et al., J. Neurosurg. 1991, 75:S14-S20). Intracranial lesions may be either focal (subdural, epidural, intracerebral bleeding; "evacuated" *vs.* "nonevacuated") or diffuse (grade I-IV). Detailed discussions of parameters used to evaluate TBI are described, for example, in Vos et al., 2002, Eur. J. Neurol. 9:207-219 and Gaetz, 2004, Clin. Neurophysiol. 115:4-18.

[0036] According to the present invention, spinal cord injury (SCI) is defined as any injury, wound, or damage to the spinal cord that results in a loss of function, such as mobility or feeling. Frequent causes of damage are trauma (*e.g.*, by car accident, gunshot, falls, etc.) or disease (polio, spina bifida, Friedreich's Ataxia, etc.). The spinal cord does not have to be severed in order for a loss of functioning to occur. In most individuals with SCI, the spinal cord is intact, but the damage results in loss of function. Besides a loss of sensation or motor function, individuals with SCI may also experience symptoms, conditions, or impairments including dysfunction of the bowel and bladder, sexual and fertility dysfunction, inability to regulate blood pressure effectively, reduced control of body temperature, inability to sweat below the level of injury, and chronic pain. A patient with SCI can have any level of SCI, as typically defined by the level of the damage (*e.g.*, at or below any of the eight cervical vertebrae or the twelve thoracic vertebrae). Very high injuries (C-1, C-2) can result in a loss of many involuntary functions including the ability to breathe, necessitating breathing aids such as mechanical ventilators or diaphragmatic pacemakers.

[0037] Methods for diagnosing spinal cord injury are well-established in the art. In the emergency room, a doctor may be able to rule out spinal cord injury by carefully inspecting an injured person, testing for sensory function and movement, and asking questions about an accident. If the injured person complains of neck pain, isn't fully awake, or has obvious signs of weakness or neurologic injury, emergency diagnostic tests may be needed. Such tests may include, X-rays, computerized tomography (CT) scan, magnetic resonance imaging (MRI), or myelography. Various neurological examinations may also be performed. The effects of SCI depend on the type of injury and the level of the injury. SCI can be generally divided into two types of injury - complete and incomplete. A complete injury means that there is no function below the level of the injury (*i.e.*, no sensation and no voluntary movement). Both sides of the body are equally affected. An incomplete injury means that there is some functioning below the primary level of the injury. A person with an incomplete injury may be able to move one limb more than another, may be able to feel parts of the body that cannot be moved, or may have more functioning on one side of the body than the other. With the advances in acute treatment of SCI, incomplete injuries are becoming more common.

[0038] The level of injury is very helpful in predicting what parts of the body might be affected by paralysis and loss of function in SCI. Cervical (neck) injuries usually result in quadriplegia. Injuries above the C-4 level may require a ventilator for the person to breathe. C-5 injuries often result in shoulder and biceps control, but no control at the wrist or hand. C-6 injuries generally yield wrist control, but no hand function. Individuals with C-7 and T-1 injuries can straighten their arms but still may have dexterity problems with the hand and fingers. Injuries at the thoracic level and below result in paraplegia, with the hands not affected. At T-1 to T-8 there is most often control of the hands, but poor trunk control as the result of lack of abdominal muscle control. Lower T-injuries (T-9 to T-12) allow good truck control and good abdominal muscle control. Sitting balance is very good. Lumbar and Sacral injuries yield decreasing control of the hip flexors and legs. Persons with tetraplegia have sustained injuries to one of the eight cervical segments of the spinal cord; those with paraplegia have lesions in the thoracic, lumbar, or sacral regions of the spinal cord.

[0039] The present invention is directed to inhibiting the physiological damage and the symptoms or conditions (disabilities, impairments) associated with such damage, that result from TBI or SCI as described in detail above. As such, it is not required that physiological damage or all effects of the condition be entirely prevented or reversed, although the effects of the present method likely extend to a significant therapeutic benefit for the patient. As such, a therapeutic benefit is not necessarily a complete prevention or cure for a particular condition or physiological damage resulting from TBI or SCI, but rather, can encompass a result which includes reducing or preventing the symptoms or physiological damage that result from TBI or SCI, reducing or preventing the occurrence of such symptoms or damage (either quantitatively or qualitatively), reducing the severity of such symptoms or physiological effects, and/or enhancing the recovery of the patient after experiencing TBI or SCI. Specifically, a composition of the present invention, when administered to a patient, preferably prevents damage associated with the brain injury or spinal cord injury and/or reduces or alleviate symptoms of or conditions associated with (resulting from) the damage, signs of the damage or even the causes of the damage, as well as enhances recovery from the damage. As such, to protect a patient from the physiological effects or symptoms resulting from TBI or SCI (or related conditions) includes both preventing or reducing the occurrence and/or severity of the effects of the damage and treating a patient in which the effects of the damage are already occurring or beginning to occur. A beneficial effect can easily be assessed by one of ordinary skill in the art and/or by a trained clinician who is treating the patient. For example, many of the methods described above for the diagnosis of TBI or SCI can be used to evaluate the patient before and after treatment using a method of the present invention to assess the success of the treatment. Preferably, there is a positive or beneficial difference in the severity or occurrence of at least

one clinical or biological score, value, or measure used to evaluate such patients in those who have been treated according to the present invention as compared to those that have not.

[0040] Inhibition of the alternative complement pathway according to the present invention for the purposes of inhibiting the physiological damage, and the symptoms or conditions (disabilities, impairments) associated with such damage, that result from TBI or SCI, can be accomplished by directly affecting the expression (transcription or translation) or biological activity of a protein in the alternative complement pathway, or by directly affecting the ability of a protein to bind to a protein in the alternative complement pathway or to otherwise contribute to the activation of complement via the alternative pathway. For example, expression of a protein refers to either the transcription of the protein or the translation of the protein. Therefore, the method of the present invention can inhibit the transcription and/or the translation of a protein in the animal that naturally expresses the protein (e.g., by administering an agent that inhibits the expression of the protein and genetically modifying an animal to have reduced protein expression). In another example, inhibition of the alternative complement pathway is defined herein as any measurable (detectable) reduction (i.e., decrease, downregulation, inhibition) of the activity of the pathway, such as by any measurable reduction in the expression and/or biological activity of a protein within the alternative complement pathway, and can include blocking or inhibiting the ability of a protein or molecule to act in the alternative complement pathway.

[0041] Methods for inhibiting the expression of a protein include, but are not limited to, administering an agent that inhibits the expression of the protein (directly or indirectly), and genetically modifying an animal to have reduced protein expression (e.g., note the *f*B-/- mice used herein). Preferably, protein expression is inhibited by administration of an agent (reagent, compound, drug) to the animal that directly inhibits protein expression. Such agents include, but are not limited to: a ribozyme or RNAi that is specific for RNA encoding the protein; a DNA binding protein or a drug that binds to a gene or RNA encoding the protein and inhibits expression of the protein; an aptamer that binds to the protein; a protein or drug that binds to the protein intracellularly and prevents secretion of the protein by the cell which expresses it; and, an isolated nucleic acid molecule that reduces expression of the protein by hybridizing under high stringency conditions to a gene encoding the protein in a cell of the animal (*e.g.*, an anti-sense nucleic acid molecule). Such compounds that selectively inhibit expression of a protein can be produced using techniques known to those of skill in the art.

[0042] Accordingly, the method described herein includes the use of a variety of agents (i.e., regulatory compounds) which, by acting directly on a protein in the alternative complement pathway, selectively inhibit the expression and/or biological activity of one or more proteins in the alternative complement pathway such that physiological damage associated with TBI or SCI is reduced in an animal. Agents useful in the present invention include, for example, proteins, nucleic acid molecules, antibodies, and compounds that are products of rational drug design (i.e., drags). Such agents are generally referred to herein as inhibitors.

[0043] According to the present invention, an inhibitor is any agent which inhibits, either by direct inhibition or competitive inhibition, the expression and/or biological activity of a protein (*e.g.*, a protein in the alternative complement pathway), and includes agents which act on factor B, factor D or properdin. In one embodiment of the present invention, inhibition of the alternative complement pathway or a protein of the alternative complement pathway is defined herein as any measurable (detectable) reduction (*i.e.*, decrease, downregulation, inhibition) of the biological activity of a protein in the alternative complement pathway. The biological activity or biological action of a protein refers to any function(s) exhibited or performed by a naturally occurring form of the protein as measured or observed *in vivo* (i.e., in the natural physiological environment of the protein) or *in vitro* (i.e., under laboratory conditions). For example, a biological activity of factor B can include, but is not limited to, binding to activated C3, solubilization of immune complexes, B cell growth factor activity, and monocyte activation. A biological activity of factor D can include, but is not limited to, catalysis of the cleavage of factor B when in complex with C3, catalysis of the formation of Ba and Bb. A biological activity of properdin can include, but is not limited to, binding to and stabilizing cell- or immune complex-bound C3bBb and stabilizing the C3/C5 convertase.

[0044] The biological activity of a protein described herein can be inhibited by directly preventing or inhibiting (reducing, decreasing) the ability of the protein to bind to and/or activate another protein (e.g., C3), thereby inhibiting downstream events resulting from such binding. Preferably, the biological activity of the alternative complement pathway is inhibited by administering an agent that inhibits at least one protein in the pathway, such agent including, but not limited to, an agent that binds to a protein in the pathway or competes with the protein in the pathway in a manner that the ability of the protein to bind to and/or activate another protein is inhibited or prevented.

[0045] Agents that inhibit a protein in the alternative complement pathway can include, but are not limited to, compounds that are products of rational drug design, natural products, and compounds having partially defined regulatory properties. A regulatory agent, including an antagonist of a given protein, can be a protein-based compound, a carbohydrate-based compound, a lipid-based compound, a nucleic acid-based compound, a natural organic compound, a synthetically derived organic compound or drug, an antibody (including antigen- binding fragments thereof), or fragments thereof. One particular type of agent useful in the present invention is an antagonist of the alternative complement pathway, including an antagonist of a protein within this pathway. According to the present invention, an "antagonist" refers to any compound that inhibits (e.g., antagonizes, reduces, decreases, blocks, reverses, or alters) the effect of a given protein. More

particularly, an antagonist is capable of acting in a manner relative to the given protein's activity, such that the biological activity of the given protein is decreased or blocked in a manner that is antagonistic (e.g., against, a reversal of, contrary to) to the natural action of the given protein. Antagonists can include, but are not limited to, an antibody or antigen binding fragment thereof, a protein, peptide, nucleic acid (including ribozymes and antisense), or a product of drug/compound/peptide design or selection that provides the antagonistic effect. For example, the present invention includes any antagonists of the natural proteins, factor B, factor D or properdin, including antibody antagonists, protein/peptide antagonists, nucleic acid antagonists, or small molecule antagonists (e.g., a small molecule inhibitor).

[0046] Regulatory agents described herein include drugs, including peptides, oligonucleotides, carbohydrates and/or synthetic organic molecules that regulate the production and/or function of one or more proteins in the alternative complement pathway. Such an agent can be obtained, for example, from molecular diversity strategies (a combination of related strategies allowing the rapid construction of large, chemically diverse molecule libraries), libraries of natural or synthetic compounds, in particular from chemical or combinatorial libraries (i.e., libraries of compounds that differ in sequence or size but that have the same building blocks) or by rational drug design. See for example, Maulik et al., 1997, Molecular Biotechnology: Therapeutic Applications and Strategies, Wiley-Liss, Inc.,

[0047] In a molecular diversity strategy, large compound libraries are synthesized, for example, from peptides, oligonucleotides, carbohydrates and/or synthetic organic molecules, using biological, enzymatic and/or chemical approaches. The critical parameters in developing a molecular diversity strategy include subunit diversity, molecular size, and library diversity. The general goal of screening such libraries is to utilize sequential application of combinatorial selection to obtain high-affinity ligands against a desired target, and then optimize the lead molecules by either random or directed design strategies. Methods of molecular diversity are described in detail in Maulik, et al., *supra.*

[0048] In a rational drug design procedure, the three-dimensional structure of a regulatory compound can be analyzed by, for example, nuclear magnetic resonance (NMR) or X-ray crystallography. This three-dimensional structure can then be used to predict structures of potential compounds, such as potential regulatory agents by, for example, computer modeling. The predicted compound structure can be used to optimize lead compounds derived, for example, by molecular diversity methods. In addition, the predicted compound structure can be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source (e.g., plants, animals, bacteria and fungi).

[0049] Various other methods of structure-based drug design are disclosed in Maulik et al., 1997, *supra.* Maulik et al. disclose, for example, methods of directed design, in which the user directs the process of creating novel molecules from a fragment library of appropriately selected fragments; random design, in which the user uses a genetic or other algorithm to randomly mutate fragments and their combinations while simultaneously applying a selection criterion to evaluate the fitness of candidate ligands; and a grid-based approach in which the user calculates the interaction energy between three dimensional receptor structures and small fragment probes, followed by linking together of favorable probe sites.

[0050] An isolated nucleic acid molecule that is useful as an agent for inhibiting a protein (or its expression) in the alternative complement pathway is an anti-sense nucleic acid molecule, a ribozyme, siRNA, or an aptamer. As used herein, an anti-sense nucleic acid molecule is defined as an isolated nucleic acid molecule that reduces expression of a protein by hybridizing under high stringency conditions to a gene encoding the protein. Such a nucleic acid molecule is sufficiently similar to the gene encoding the protein that the molecule is capable of hybridizing under high stringency conditions to the coding or complementary strand of the gene or RNA encoding the natural protein. RNA interference (RNAi) is a process whereby double stranded RNA, and in mammalian systems, short interfering RNA (siRNA), is used to inhibit or silence expression of complementary genes. In the target cell, siRNA are unwound and associate with an RNA induced silencing complex (RISC), which is then guided to the mRNA sequences that are complementary to the siRNA, whereby the RISC cleaves the mRNA. A ribozyme is an RNA segment that functions by binding to the target RNA moiety and inactivate it by cleaving the phosphodiester backbone at a specific cutting site. Aptamers are short strands of synthetic nucleic acids (usually RNA but also DNA) selected from randomized combinatorial nucleic acid libraries by virtue of their ability to bind to a predetermined specific target molecule with high affinity and specificity. Aptamers assume a defined three-dimensional structure and are capable of discriminating between compounds with very small differences in structure.

[0051] A gene includes regulatory regions that control production of the protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. The genes encoding various proteins of the alternative complement pathway, including factor B, factor D or properdin, have been identified and are known in the art. An isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis.

[0052] As used herein, reference to hybridization conditions refers to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for

example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, 1989. Sambrook et al., ibid., (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are disclosed, for example, in Meinkoth et al., 1984, Anal. Biochem. 138, 267-284; Meinkoth et al., *ibid*., is incorporated by reference herein in its entirety.

[0053] In a preferred embodiment of the present invention, the agent used for inhibiting a protein of the alternative complement pathway for the purpose of inhibiting the physiological damage that results from TBI or SCI (and the symptoms or conditions (disabilities, impairments) associated with such damage), is an antibody or an antigen binding fragment thereof. Similarly, an antigen binding polypeptide is also particularly preferred for use in the present invention. In one aspect, the antibody selectively binds to the protein of the alternative complement pathway in a manner such that the protein is inhibited or prevented from binding to another protein with which it normally (under natural or physiological conditions) interacts. In another aspect, the antibody selectively binds to the protein in a manner such that the protein is inhibited or prevented from activating another protein with which it normally interacts, even though the protein may at least partially bind to the other protein. Particularly preferred antibodies and antigen binding fragments thereof for use in selective inhibition of the alternative complement pathway for the purpose of inhibiting the physiological damage that results from TBI or SCI are described in detail below (e.g., the factor B antibodies described herein, and particularly, the mAb1379 antibody described in detail herein).

[0054] Preferably, an antibody or antigen binding fragment thereof useful in the present invention binds to a protein selected from factor B, factor D or properdin. Most preferably, the invention includes an antibody or antigen binding fragment thereof that binds to factor B, and its use for the purpose of inhibiting the physiological damage that results from TBI or SCI. Antibodies (and antigen binding fragments thereof) that selectively bind to factor B and inhibit the alternative complement pathway according to the invention are described and exemplified in detail herein. In one embodiment, the antibody or antigen binding fragment thereof binds to a conserved binding surface or epitope of such a protein (e.g., factor B) that is conserved among animal species, and particularly mammalian, species (i.e., the antibody is cross-reactive with the protein from two or more different mammalian species). In particular, the present invention includes an antibody that binds to factor B from at least two, and preferably, several different mammalian species, including, but not limited to, human, non-human primate, mouse, rat, pig, horse and rabbit. Preferably, the present invention includes an antibody that binds to factor B from human and at least one additional animal species, and preferably, at least one additional mammalian species, including, but not limited to, non-human primate, mouse, rat, pig, horse and rabbit. In one embodiment, the antibody or antigen binding fragment thereof binds to the third short consensus repeat (SCR) of factor B. In one embodiment, the antibody or antigen binding fragment thereof binds to a region of factor B that prevents the cleavage of factor B by factor D. In one embodiment, the antibody is a monoclonal antibody. In one embodiment, the antibody is the antibody referred to herein as 1379 (*i.e.,* the antibody produced by the hybridoma cell line of the same number, also having ATCC Deposit Designation PTA-6230), or an antigen binding fragment thereof.

[0055] The hybridoma described herein as 1379 (or mAb1379) was deposited on September 21, 2004, with the American Type Culture Collection (ATCC, located at 10801 University Blvd, Manassas, VA 20110-2209), under the terms of the Budapest Treaty on the International Recognition of The Deposit of Microorganisms For the Purposes of Patent Procedure, and has received ATCC Deposit Designation PTA-6230.

[0056] As described herein, the minimum size of a protein, portion of a protein (e.g. a fragment, portion, domain, etc.), or region or epitope of a protein, is a size sufficient to serve as an epitope or conserved binding surface for the generation of an antibody or as a target in an *in vitro* assay. The protein may be at least about 4, 5, 6, 7 or 8 amino acids in length (e.g., suitable for an antibody epitope or as a detectable peptide in an assay), or at least about 25 amino acids in length, or at least about 50 amino acids in length, or at least about 100 amino acids in length, or at least about 150 amino acids in length, and so on, in any length between 4 amino acids and up to the full length of a protein or portion thereof or longer, in whole integers (e.g., 8, 9, 10,...25, 26,...500, 501,...).

[0057] The nucleotide sequence for the gene and coding region encoding human factor B and other complement proteins, as well as the amino acid sequence of such proteins, are well known in the art. For example, the gene encoding human factor B and other complement proteins is found in NCBI Database Accession No. NG_000013. The coding sequence for factor B is found in NCBI Database Accession No. NM_001710 and the amino acid sequence for factor B preproprotein is found in NCBI Database Accession No. NP_001701 or P00751. The amino acid sequence for NCBI Database Accession No. P00751, which is a human preproprotein factor B sequence, is represented herein by SEQ ID NO:1. Sequences from other animal species are also known in the art. By way of comparison, in the mouse factor B sequence (e.g., see NCBI Database Accession No. P04186, represented herein by SEQ ID NO:6), the third SCR domain is located at positions 160-217 of this 761 amino acid preprotein, and the mature murine factor B protein spans positions 23-761 of SEQ ID NO:6. The coding sequence for human factor D is found in NCBI Database Accession No. NM_001928.2 and the amino acid sequence for human factor D preproprotein is found in NCBI Database Accession No. NP_001919 (represented herein as SEQ ID NO:7). The coding sequence for human properdin is found in NCBI Database Accession No. NM_002621.1 and the amino acid sequence for human properdin is found in NCBI Database Accession No.

NP_002612 (represented herein by SEQ ID NO:8).

[0058] The human factor B preprotein represented by SEQ ID NO:1 is a 764 amino acid protein with a signal peptide spanning from amino acid positions 1-25. The mature chain of factor B corresponds to positions 26-764 of SEQ ID NO:1 and is represented herein by SEQ ID NO:2. The three SCR regions of human factor B are represented herein by SEQ ID NO:3 (SCR1, also known as Sushi 1, spanning from about position 35 to about position 100 of SEQ ID NO:1 or from about position 5 to about position 75 of SEQ ID NO:2), SEQ ID NO:4 (SCR2, also known as Sushi 2, spanning from about position 101 to about position 160 of SEQ ID NO:1 or from about position 76 to about position135 of SEQ ID NO:2), and SEQ ID NO:5 (SCR3, also known as Sushi 3, spanning from about position 163 to about position 220 of SEQ ID NO:1 or from about position 138 to about position 195 of SEQ ID NO:2).

[0059] Based on the epitope mapping of factor B using the fragments described by Hourcade, 1995, *J. Biol Chem*., in one preferred embodiment, an anti-factor B antibody useful in the present invention preferably binds to an epitope or conserved binding surface within or containing a part of the third SCR domain, and more preferably, to an epitope of human factor B that includes at least a portion of the sequence comprising from about position Tyr139 to about position Ser185 with respect to the mature factor B protein (SEQ ID NO:2), to an epitope of human factor B that includes at least a portion of the sequence comprising from about position Tyr139 to about position Ser141 with respect to the mature factor B protein (SEQ ID NO:2), to an epitope of human factor B that includes at least a portion of the sequence comprising from about position Glu182 to about position Ser185 with respect to the mature factor B protein (SEQ ID NO:2), to an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising any one or more of the following positions or their equivalent positions in a non-human factor B sequence: Tyr139, Cys 140, Ser141, Glu182, Gly184, or Ser185, or to an epitope of factor B that includes at least a portion of the equivalent positions with respect to non-human animal species. One of skill in the art can readily align the sequence of human factor B with the sequence of factor B from another animal species and determine the positions of the SCR regions and the specific portions of the third SCR regions corresponding to the amino acid positions above. For example, two specific sequences can be aligned to one another using BLAST 2 sequence as described in Tatusova and Madden, (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250.

[0060] Based on additional epitope modeling and mapping of an exemplary antibody useful in the invention, in another preferred embodiment, an anti-factor B antibody useful in the present invention preferably binds to an epitope (conserved binding surface) within or containing a part or portion of the third SCR domain of factor B that includes at least one or more of the following amino acid positions, with respect to SEQ ID NO:2, or their equivalent positions in a non-human factor B sequence: A137, Y139, S141, E182, S185, T189, E190, and S192. In one aspect of the invention, the epitope is within or containing a part of portion of the third SCR domain of factor B that includes all or substantially all of (at least five, six, or seven of the following amino acid positions of SEQ ID NO:2, or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192. In yet another aspect, the epitope recognized by an anti-factor B antibody useful in the present invention is within or contains a part or portion of the third SCR domain of factor B consisting of the following amino acid positions of SEQ ID NO:2, or their equivalent positions in a non-human factor B sequence: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192.

[0061] In one embodiment, the epitope recognized by a factor B antibody useful in the invention can also be defined more particularly as being non-linear epitope located within the three-dimensional structure of a portion of the third SCR domain of factor B. The portion that contains the epitope is the three-dimensional structure of factor B that is defined by at substantially all of (e.g., at least about 90% of) amino acid positions Ala137-Ser192 of SEQ ID NO:2, or equivalent positions in a non-human factor B sequence, when such sequence is conformationally arranged as it occurs in the natural full-length factor B sequence. A model of the three-dimensional structure of factor B, which illustrates an epitope for mAb1379 is illustrated in Fig. 4 and Fig. 5, for example. As used herein, the "three dimensional structure" or "tertiary structure" of a protein refers to the arrangement of the components of the protein in three dimensions. Such term is well known to those of skill in the art. As used herein , the term "model" refers to a representation in a tangible medium of the three dimensional structure of a protein, polypeptide or peptide. For example, a model can be a representation of the three dimensional structure in an electronic file, on a computer screen, on a piece of paper (i.e., on a two dimensional medium), and/or as a ball-and-stick figure.

[0062] As described herein, an "epitope" of a given protein or peptide or other molecule is generally defined, with regard to antibodies, as a part of or site on a larger molecule to which an antibody or antigen-binding fragment thereof will bind, and against which an antibody will be produced. The term epitope can be used interchangeably with the term "antigenic determinant", "antibody binding site", or "conserved binding surface" of a given protein or antigen. More specifically, an epitope can be defined by both the amino acid residues involved in antibody binding and also by their conformation in three dimensional space (e.g., a conformational epitope or the conserved binding surface). An epitope can be included in peptides as small as about 4-6 amino acid residues, or can be included in larger segments of a protein, and need not be comprised of contiguous amino acid residues when referring to a three dimensional structure of an epitope, particularly with regard to an antibody- binding epitope. Antibody-binding epitopes are frequently conformational epitopes rather than a sequential epitope (i.e., linear epitope), or in other words, an epitope defined by amino

acid residues arrayed in three dimensions on the surface of a protein or polypeptide to which an antibody binds. As mentioned above, the conformational epitope is not comprised of a contiguous sequence of amino acid residues, but instead, the residues are perhaps widely separated in the primary protein sequence, and are brought together to form a binding surface by the way the protein folds in its native conformation in three dimensions. The epitope recognized by the mAb1379 is a conformational epitope that is not a linear epitope.

[0063] One of skill in the art can identify and/or assemble conformational epitopes and/or sequential epitopes using known techniques, including mutational analysis (e.g., site-directed mutagenesis); protection from proteolytic degradation (protein footprinting); mimotope analysis using, e.g., synthetic peptides and pepscan, BIACORE or ELISA; antibody competition mapping; combinatorial peptide library screening; matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry; or three-dimensional modeling (e.g., using any suitable software program, including, but not limited to, MOLSCRIPT 2.0 (Avatar Software AB, Heleneborgsgatan 21C, SE-11731 Stockholm, Sweden), the graphical display program O (Jones et. al., Acta Crystallography, vol. A47, p. 110, 1991), the graphical display program GRASP, or the graphical display program INSIGHT). For example, one can use molecular replacement or other techniques and the known three-dimensional structure of a related protein to model the three-dimensional structure of factor B and predict the conformational epitope of antibody binding to this structure. Indeed, one can use one or any combination of such techniques to define the antibody binding epitope. Figs. 4 and 5 illustrate the use of three-dimensional modeling, combined with information from mimotope analysis and mutational analysis, to identify the epitope of a factor B antibody useful in the present invention.

[0064] As used herein, the term "selectively binds to" refers to the specific binding of one protein to another (e.g., an antibody, fragment thereof, or binding partner to an antigen), wherein the level of binding, as measured by any standard assay (e.g., an immunoassay), is statistically significantly higher than the background control for the assay. For example, when performing an immunoassay, controls typically include a reaction well/tube that contain antibody or antigen binding fragment alone (i.e., in the absence of antigen), wherein an amount of reactivity (e.g., non-specific binding to the well) by the antibody or antigen binding fragment thereof in the absence of the antigen is considered to be background. Binding can be measured using a variety of methods standard in the art, including, but not limited to: Western blot, immunoblot, enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry.

[0065] One embodiment of the present invention includes the use of an antibody or antigen binding fragment thereof that is a competitive inhibitor of the binding of factor B to the anti-factor B antibody (e.g., monoclonal antibody 1379) to inhibit physiological damage and effects associated with TBI or SCI. According to the present invention, a competitive inhibitor of factor B binding to an anti-factor B antibody of the present invention is an inhibitor (e.g., another antibody or antigen binding fragment or polypeptide) that binds to factor B at the same or similar epitope as the known anti-factor B antibody of the present invention (e.g., mAb 1379) such that binding of the known anti-factor B antibody to factor B is inhibited. A competitive inhibitor may bind to the target (e.g., factor B) with a greater affinity for the target than the anti-factor B antibody. A competitive inhibitor can be used in a manner similar to that described herein for the anti-factor B antibody 1379 (e.g., to inhibit the alternative complement pathway, to inhibit physiological damage or effects caused by TBI or SCI). For example, one embodiment of the invention relates to the use of an isolated antibody or antigen binding fragment thereof that specifically binds to factor B, wherein the antibody or fragment thereof competitively inhibits mAb1379 for specific binding to factor B, and wherein, when the antibody or fragment thereof binds to factor B, the alternative complement pathway is inhibited or alternatively, the ability of mAb1379 to inhibit the alternative complement pathway is inhibited. Another embodiment relates to the use of an isolated antibody or fragment thereof that specifically binds to factor B, wherein the isolated antibody or fragment thereof competitively inhibits a second antibody or fragment thereof for specific binding to factor B, and wherein the second antibody or fragment thereof binds to the third SCR domain of factor B.

[0066] Competition assays can be performed using standard techniques in the art (e.g., competitive ELISA or other binding assays). For example, competitive inhibitors can be detected and quantitated by their ability to inhibit the binding of factor B to a known, labeled anti-factor B antibody (e.g., the mAb 1379). Antibody-antibody competition assays in the presence of human factor B are described in U.S. Patent Application Publication No. 2005-0260198-A1 and in PCT Publication No. WO 2005/077417, *supra.* Competitive inhibitors of the binding of factor B to anti-factor B 1379 are also described in U.S. Patent Application Publication No. 2005-0260198-A1 and in PCT Publication No. WO 2005/077417, *supra.*

[0067] Antibodies described herein are characterized in that they comprise immunoglobulin domains and as such, they are members of the immunoglobulin superfamily of proteins. Generally speaking, an antibody molecule comprises two types of chains. One type of chain is referred to as the heavy or H chain and the other is referred to as the light or L chain. The two chains are present in an equimolar ratio, with each antibody molecule typically having two H chains and two L chains. The two H chains are linked together by disulfide bonds and each H chain is linked to a L chain by a

disulfide bond. There are only two types of L chains referred to as lambda (λ) and kappa (κ) chains. In contrast, there are five major H chain classes referred to as isotypes. The five classes include immunoglobulin M (IgM or μ), immunoglobulin D (IgD or δ), immunoglobulin G (IgG or X), immunoglobulin A (IgA or α), and immunoglobulin E (IgE or ε). The distinctive characteristics between such isotypes are defined by the constant domain of the immunoglobulin and are discussed in detail below. Human immunoglobulin molecules comprise nine isotypes, IgM, IgD, IgE, four subclasses of IgG including IgG1 (γ1), IgG2 (γ2), IgG3 (γ3) and IgG4 (γ4), and two subclasses of IgA including IgA1 (α1) and IgA2 (α2). In humans, IgG subclass 3 and IgM are the most potent complement activators (classical complement system), while IgG subclass 1 and to an even lesser extent, 2, are moderate to low activators of the classical complement system. IgG4 subclass does not activate the complement system (classical or alternative). The only human immunoglobulin isotype known to activate the alternative complement system is IgA. In mice, the IgG subclasses are IgG1, IgG2a, IgG2b and IgG3. Murine IgG1 does not activate complement, while IgG2a, IgG2b and IgG3 are complement activators.

[0068] Each H or L chain of an immunoglobulin molecule comprises two regions referred to as L chain variable domains (V_L domains) and L chain constant domains (C_L domains), and H chain variable domains (V_H domains) and H chain constant domains (C_H domains). A complete C_H domain comprises three sub-domains (CH1, CH2, CH3) and a hinge region. Together, one H chain and one L chain can form an arm of an immunoglobulin molecule having an immunoglobulin variable region. A complete immunoglobulin molecule comprises two associated (*e.g.*, di-sulfide linked) arms. Thus, each arm of a whole immunoglobulin comprises a V_{H+L} region, and a C_{H+L} region. As used herein, the term "variable region" or "V region" refers to a V_{H+L} region (also known as an Fv fragment), a V_L region or a V_H region. Also as used herein, the term "constant region" or "C region" refers to a C_{H+L} region, a C_L region or a C_H region.

[0069] Limited digestion of an immunoglobulin with a protease may produce two fragments. An antigen binding fragment is referred to as an Fab, an Fab', or an $F(ab')_2$ fragment. A fragment lacking the ability to bind to antigen is referred to as an Fc fragment. An Fab fragment comprises one arm of an immunoglobulin molecule containing a L chain (V_L + C_L domains) paired with the V_H region and a portion of the C_H region (CH1 domain). An Fab' fragment corresponds to an Fab fragment with part of the hinge region attached to the CH1 domain. An $F(ab')_2$ fragment corresponds to two Fab' fragments that are normally covalently linked to each other through a di-sulfide bond, typically in the hinge regions.

[0070] The C_H domain defines the isotype of an immunoglobulin and confers different functional characteristics depending upon the isotype. For example, μ constant regions enable the formation of pentameric aggregates of IgM molecules and α constant regions enable the formation of dimers.

[0071] The antigen specificity of an immunoglobulin molecule is conferred by the amino acid sequence of a variable, or V, region. As such, V regions of different immunoglobulin molecules can vary significantly depending upon their antigen specificity. Certain portions of a V region are more conserved than others and are referred to as framework regions (FW regions). In contrast, certain portions of a V region are highly variable and are designated hypervariable regions. When the V_L and V_H domains pair in an immunoglobulin molecule, the hypervariable regions from each domain associate and create hypervariable loops that form the antigen binding sites. Thus, the hypervariable loops determine the specificity of an immunoglobulin and are termed complementarity-determining regions (CDRs) because their surfaces are complementary to antigens.

[0072] Further variability of V regions is conferred by combinatorial variability of gene segments that encode an immunoglobulin V region. Immunoglobulin genes comprise multiple germline gene segments which somatically rearrange to form a rearranged immunoglobulin gene that encodes an immunoglobulin molecule. V_L regions are encoded by a L chain V gene segment and J gene segment (joining segment). V_H regions are encoded by a H chain V gene segment, D gene segment (diversity segment) and J gene segment (joining segment).

[0073] Both a L chain and H chain V gene segment contain three regions of substantial amino acid sequence variability. Such regions are referred to as L chain CDR1, CDR2 and CDR3, and H chain CDR1, CDR2 and CDR3, respectively. The length of an L chain CDR1 can vary substantially between different V_L regions. For example, the length of CDR1 can vary from about 7 amino acids to about 17 amino acids. In contrast, the lengths of L chain CDR2 and CDR3 typically do not vary between different V_L regions. The length of a H chain CDR3 can vary substantially between different V_H regions. For example, the length of CDR3 can vary from about 1 amino acid to about 20 amino acids. Each H and L chain CDR region is flanked by FW regions.

[0074] Other functional aspects of an immunoglobulin molecule include the valency of an immunoglobulin molecule, the affinity of an immunoglobulin molecule, and the avidity of an immunoglobulin molecule. As used herein, affinity refers to the strength with which an immunoglobulin molecule binds to an antigen at a single site on an immunoglobulin molecule (*i.e.*, a monovalent Fab fragment binding to a monovalent antigen). Affinity differs from avidity, which refers to the sum total of the strength with which an immunoglobulin binds to an antigen. Immunoglobulin binding affinity can be measured using techniques standard in the art, such as competitive binding techniques, equilibrium dialysis or BIAcore methods. As used herein, valency refers to the number of different antigen binding sites per immunoglobulin molecule (*i.e.*, the number of antigen binding sites per antibody molecule of antigen binding fragment). For example, a monovalent immunoglobulin molecule can only bind to one antigen at one time, whereas a bivalent immunoglobulin molecule can bind to two or more antigens at one time, and so forth. Both monovalent and bivalent antibodies that selectively bind to proteins

of the alternative complement pathway are encompassed herein.

[0075] The antibody may be a bi- or multi-specific antibody. A bi-specific (or multi-specific) antibody is capable of binding two (or more) antigens, as with a divalent (or multivalent) antibody, but in this case, the antigens are different antigens (*i.e.*, the antibody exhibits dual or greater specificity). For example, an antibody that selectively binds to a protein in the alternative complement pathway according to the present invention (e.g., an anti- factor B antibody as described herein) can be constructed as a bi-specific antibody, wherein the second antigen binding specificity is for a desired target. Therefore, one bi-specific antibody encompassed by the present invention includes an antibody having: (a) a first portion (e.g., a first antigen binding portion) which binds to a protein in the alternative complement pathway (e.g., factor B); and (b) a second portion which binds to a cell surface molecule expressed by a cell. In this embodiment, the second portion can bind to any cell surface molecule. One preferred cell surface molecule is a receptor or ligand, so that the antibody is targeted to a particular cell or tissue type and/or to a particular site in an animal to which the antibody is delivered. In one embodiment, the second antigen binding specificity is for a complement receptor. A particularly preferred complement receptor includes, but is not limited to, complement receptor type 2 (CR2). Antibodies that selectively bind to CR2 and could therefore be used in this embodiment of the invention are described, for example, in U.S. Patent No. 6,820,011.

[0076] In one example, antibodies may include humanized antibodies. Humanized antibodies are molecules having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining inimunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable regions fused onto human constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domains. Humanized antibodies can be produced, for example, by modeling the antibody variable domains, and producing the antibodies using genetic engineering techniques, such as CDR grafting (described below). A description various techniques for the production of humanized antibodies is found, for example, in Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-55; Whittle et al. (1987) Prot. Eng. 1:499-505; Co et al. (1990) J. Immunol. 148:1149-1154; Co et al. (1992) Proc. Natl. Acad. Sci. USA 88:2869-2873; Carter et al. (1992) Proc. Natl Acad. Sci. 89:4285-4289; Routledge et al. (1991) Eur. J. Immunol. 21:2717-2725 and PCT Patent Publication Nos. WO 91/09967; WO 91/09968 and WO 92/113831.

[0077] Isolated antibodies described herein can include serum containing such antibodies, or antibodies that have been purified to varying degrees. Whole antibodies of the present invention can be polyclonal or monoclonal. Alternatively, functional equivalents of whole antibodies, such as antigen binding fragments in which one or more antibody domains are truncated or absent (*e.g.*, Fv, Fab, Fab', or F(ab)2 fragments), as well as genetically-engineered antibodies or antigen binding fragments thereof, including single chain antibodies, humanized antibodies (discussed above), antibodies that can bind to more than one epitope (*e.g.*, bi-specific antibodies), or antibodies that can bind to one or more different antigens (*e.g.*, bi- or multi-specific antibodies), may also be employed in the invention.

[0078] Genetically engineered antibodies described herein include those produced by standard recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and/or constant regions. Particular examples include, chimeric antibodies, where the V_H and/or V_L domains of the antibody come from a different source as compared to the remainder of the antibody, and CDR grafted antibodies (and antigen binding fragments thereof), in which at least one CDR sequence and optionally at least one variable region framework amino acid is (are) derived from one source and the remaining portions of the variable and the constant regions (as appropriate) are derived from a different source. Construction of chimeric and CDR-grafted antibodies are described, for example, in European Patent Applications: EP-A 0194276, EP-A 0239400, EP-A 0451216 and EP-A 0460617.

[0079] In one example, chimeric antibodies are produced comprising antibody variable domains that bind to a protein in the alternative complement pathway (e.g., factor B) and fused to these domains, a protein that serves as a second targeting moiety. For example, the targeting moiety can include a protein that is associated with the cell or tissue to be targeted or with a particular system in the animal. For example, the targeting moiety can be a selectin or a portion of a complement receptor. One preferred complement receptor to use in this aspect of the invention includes complement receptor type 2 (CR2). The use of CR2 and portions thereof in a fusion or chimeric protein (e.g., as a delivery system) is described in detail in U.S. Patent No. 6,820,011.

[0080] Generally, in the production of an antibody, a suitable experimental animal, such as, for example, but not limited to, a rabbit, a sheep, a hamster, a guinea pig, a mouse, a rat, or a chicken, is exposed to an antigen against which an antibody is desired. Typically, an animal is immunized with an effective amount of antigen that is injected into the animal. An effective amount of antigen refers to an amount needed to induce antibody production by the animal. The animal's immune system is then allowed to respond over a pre-determined period of time. The immunization process can be repeated until the immune system is found to be producing antibodies to the antigen. In order to obtain polyclonal antibodies specific for the antigen, serum is collected from the animal that contains the desired antibodies (or in the case of a chicken, antibody can be collected from the eggs). Such serum is useful as a reagent. Polyclonal antibodies can be further purified from the serum (or eggs) by, for example, treating the serum with ammonium sulfate.

[0081] Monoclonal antibodies may be produced according to the methodology of Kohler and Milstein (Nature

256:495-497, 1975). For example, B lymphocytes are recovered from the spleen (or any suitable tissue) of an immunized animal and then fused with myeloma cells to obtain a population of hybridoma cells capable of continual growth in suitable culture medium. Hybridomas producing the desired antibody are selected by testing the ability of the antibody produced by the hybridoma to bind to the desired antigen.

[0082] A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein or peptide (e.g., a factor B protein or peptide including domains thereof) to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly. For example, once a cell line, for example a hybridoma, expressing an antibody according to the invention has been obtained, it is possible to clone therefrom the cDNA and to identify the variable region genes encoding the desired antibody, including the sequences encoding the CDRs. From here, antibodies and antigen binding fragments according to the invention may be obtained by preparing one or more replicable expression vectors containing at least the DNA sequence encoding the variable domain of the antibody heavy or light chain and optionally other DNA sequences encoding remaining portions of the heavy and/or light chains as desired, and transforming/transfecting an appropriate host cell, in which production of the antibody will occur. Suitable expression hosts include bacteria, (for example, an *E. coli* strain), fungi, (in particular yeasts, *e.g.* members of the genera *Pichia, Saccharomyces,* or *Kluyveromyces,*) and mammalian cell lines, *e.g.* a non-producing myeloma cell line, such as a mouse NSO line, or CHO cells. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operably linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well known and routinely used. For example, basic molecular biology procedures are described by Maniatis et al. (Molecular Cloning, Cold Spring Harbor Laboratory, New York, 1989); DNA sequencing can be performed as described in Sanger et al. (PNAS 74, 5463, (1977)) and the Amersham International plc sequencing handbook; and site directed mutagenesis can be carried out according to the method of Kramer et al. (Nucl. Acids Res. 12, 9441, (1984)) and the Anglian Biotechnology Ltd. handbook. Additionally, there are numerous publications, including patent specifications, detailing techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors and transformation of appropriate cells, for example as reviewed by Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews (ed. Tombs, M P, 10), Chapter 1, 1992, Intercept, Andover, UK) and in the aforementioned European Patent Applications.

[0083] Alternative methods, employing, for example, phage display technology (see for example US 5969108, US 5565332, US 5871907, US 5858657) or the selected lymphocyte antibody method of US 5627052 may also be used for the production of antibodies and/or antigen fragments of the invention, as will be readily apparent to the skilled individual.

[0084] Also described is the use of non-antibody polypeptides, sometimes referred to as antigen binding partners or antigen binding polypeptides, that have been designed to bind selectively to and cause the neutralization or inhibition of a protein according to the present invention. Examples of the design of such polypeptides, which possess a prescribed ligand specificity are given in Beste et al. (Proc. Natl. Acad. Sci. 96:1898-1903, 1999).

[0085] The present invention also includes a formulation or composition for reducing physiological damage associated with TBI or SCI. The formulation comprises: (a) any one or more inhibitors of the alternative complement pathway as described herein (e.g., an anti-factor B antibody described herein); and (b) at least one pharmaceutically acceptable carrier.

[0086] The formulation or composition can include one or more additional agents, such as another agent that is suitable for treating at least one symptom of, or physiological damage associated with, TBI (*e.g.*, osmotic drugs, sedatives, analgesics, muscle relaxants, barbiturates, etc.). In addition, the formulation can be administered to a patient in conjunction with another therapy or protocol that is used to treat or ameliorate damage associated with TBI. Such therapies or protocols include, but are not limited to: reduction of mass lesions by surgical evacuation of intracranial hematomas; the reduction of brain swelling with osmotic drugs (*e.g.*, mannitol); the therapeutic drainage of cerebrospinal fluid (CSF) through intraventricular catheters; achievement and maintenance of adequate gas exchange and circulatory stability; prevention of hypoxemia and hypercarbia; repeated CT scans for detection of delayed secondary intracranial pathology; profound sedation and analgesia to avoid stress and pain; achievement and maintenance of optimal CPP (> 70 mmHg) and cerebral oxygen balance; avoidance of hyperthermia (< 38°C); prevention of hyperglycemia and hyponatremia; prevention of routinely performed head elevation; prevention of stress ulcers and maintenance of gut mucosal integrity; prophylaxis for complicating factors (*e.g.* pneumonia or meningitis); intracranial pressure (ICP)-targeted therapy (*e.g.*, deepening of sedation, analgesia, muscle relaxation; CSF drainage through ventricular catheters; moderate hyperventilation under certain circumstances; osmotherapy; moderate hypothermia ($\pm$ 34°C); and/or barbiturate coma); and/or gas-enabled (CO) attenuation of neuroinflammation. Various treatments for TBI are well known in the art and are described, for example, in Royo et al., 2003, Current Opin. Pharmacol. 3:27-32; Dutton and McCunn, 2003, Current Opin. Crit. Care 9:503-509; Elf et al., 2003, Eur. J. Trauma 29:74-80; Ghajar et al., 2000, Lancet 356:923-929.

[0087] The formulation or composition can include one or more additional agents, such as another agent that is suitable for treating at least one symptom of, or physiological damage associated with, SCI (e.g., steroids, such as methylpred-

nisolone). In addition, the formulation can be administered to a patient in conjunction with another therapy or protocol that is used to treat or ameliorate damage associated with SCI. Such therapies or protocols include, but are not limited to: immobilization of the spine; decompression surgery; surgery to stabilize the vertebrae; surgery to realign the vertebrae; traction. Various treatments for SCI are well known in the art and are described, for example, in Ramer et al., 2005, Spinal Cord 43(3):134-61.

[0088] According to the present invention, a "pharmaceutically acceptable carrier" includes pharmaceutically acceptable excipients and/or pharmaceutically acceptable delivery vehicles, which are suitable for use in the administration of a formulation or composition to a suitable *in vivo* site. A suitable *in vivo* site is preferably any site wherein the alternative complement pathway can be inhibited, but in one preferred embodiment, is in the brain tissue of a patient that has or is at risk of developing, physiological damage associated with TBI or SCI. Preferred pharmaceutically acceptable carriers are capable of maintaining an agent used in a formulation of the invention in a form that, upon arrival of the agent at the target site in a patient, the agent is capable of acting on its target (e.g., a protein that is a component of the alternative complement pathway), preferably resulting in a therapeutic benefit to the patient.

[0089] Suitable excipients of the present invention include excipients or formularies that transport or help transport, but do not specifically target a composition to a cell or tissue (also referred to herein as non-targeting carriers). Examples of pharmaceutically acceptable excipients include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity. Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, m- or o-cresol, formalin and benzol alcohol. Formulations of the present invention can be sterilized by conventional methods and/or lyophilized.

[0090] One type of pharmaceutically acceptable carrier includes a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises an agent of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other suitable carriers include any carrier that can be bound to or incorporated with the agent that extends that half-life of the agent to be delivered. Such a carrier can include any suitable protein carrier or even a fusion segment that extends the half-life of a protein when delivered *in vivo.* Suitable delivery vehicles have been previously described herein, and include, but are not limited to liposomes, viral vectors or other delivery vehicles, including ribozymes. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. A delivery vehicle as described herein can be modified to target to a particular site in a patient, thereby targeting and making use of an inhibitory agent at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a targeting agent capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Other suitable delivery vehicles include gold particles, poly-L-lysine/DNA-molecular conjugates, and artificial chromosomes.

[0091] A pharmaceutically acceptable carrier which is capable of targeting is referred to as a "targeting delivery vehicle." Targeting delivery vehicles of the present invention are capable of delivering a formulation, including an inhibitory agent, to a target site in a patient. A "target site" refers to a site in a patient to which one desires to deliver a therapeutic formulation. For example, a target site can be any cell or tissue that is targeted by direct injection or delivery using liposomes, viral vectors or other delivery vehicles, including ribozymes. A delivery vehicle as described herein can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell or tissue type (*e.g.*, to the brain or to the central nervous system). Specifically, targeting refers to causing a delivery vehicle to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. Particularly useful examples include any ligands that are associated with the complement pathway (e.g., CR2, C3, C3d, C3dg, iC3b, C3b) or any ligands (*e.g.*, selectins) that are associated with the cell type, tissue type, or site in the animal to be treated.

[0092] Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics. In one example, a targeting delivery vehicle can be a formulation that allows a compound to cross the blood-brain barrier.

[0093] One delivery vehicle useful for a variety of administration routes and agents is a liposome. A liposome is capable

of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule described in the present invention to a preferred site in the animal. A liposome, according to the present invention, comprises a lipid composition that is capable of delivering a nucleic acid molecule or other compound to a particular, or selected, site in an animal. A liposome according to the methods described herein comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver a nucleic acid molecule into a cell. Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes typically used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Complexing a liposome with a nucleic acid molecule or inhibitory agent of the present invention can be achieved using methods standard in the art.

[0094] Another delivery vehicle comprises a viral vector. A viral vector includes an isolated nucleic acid molecule useful in the method of the present invention, in which the nucleic acid molecules are packaged in a viral coat that allows entrance of DNA into a cell. A number of viral vectors can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, lentiviruses, adeno-associated viruses and retroviruses.

[0095] Agents and formulations, as described herein can be administered to any animal or patient, and preferably to humans. According to the present invention, administration of an agent or formulation is useful to inhibit any symptom of physiological damage associated with TBI, SCI, or similar or related conditions. Patients whom are suitable candidates for the method of the present invention include, but are not limited to, patients that have, or are at risk of developing (e.g., are likely or predicted to develop), physiological damage to the brain or spinal cord and conditions related to this damage, as a result of injury (including traumatic injury) or disease.

[0096] In accordance with the methods described herein, determination of acceptable protocols to administer an agent or a composition including the agent, including the route of administration and the effective amount of an agent to be administered to an animal, can be accomplished by those skilled in the art. An agent or composition as described herein can be administered *in vivo* or *ex vivo.* Suitable *in vivo* routes of administration can include, but are not limited to, oral, nasal, inhaled, topical, intratracheal, transdermal, rectal, brain (*e.g.*, intracranial), spinal (*e.g.*, intraspinal or to the epidural space of the spinal cord), and parenteral routes. Preferred parenteral routes can include, but are not limited to, subcutaneous, intradermal, intravenous, intramuscular, and intraperitoneal routes. Preferred topical routes include inhalation by aerosol (i.e., spraying) or topical surface administration to the skin of an animal. Preferably, an agent is administered by systemic routes (e.g., intraperitoneal, intravenous), with intravenous administration being particularly preferred, or by administration to the brain, spinal cord, or the epidural space of the spinal cord. For traumatic brain injury, administration by intravenous administration or to the brain is preferred. For spinal cord injury, administration by intravenous administration, spinal cord administration, or administration to the epidural space of the spinal cord is preferred. *Ex vivo* refers to performing part of the administration step outside of the patient.

[0097] Techniques for administration of agents and compositions to the brain and central nervous system include, but art not limited to, intravenous administration, intraperitoneal administration, intraarterial delivery with blood-brain barrier disruption, continuous infusion of drugs through the brain using convection-enhanced delivery methods, implantation, intrathecal infusion, intraventricular administration, interstitial administration, and intraspinal administration. Intravenous, intraperitoneal, intramuscular, and intraspinal administrations can be performed using methods standard in the art. Aerosol (inhalation) delivery can be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992. Carriers suitable for aerosol delivery are described above. Devices for delivery of aerosolized formulations include, but are not limited to, pressurized metered dose inhalers (MDI), dry powder inhalers (DPI), and metered solution devices (MSI), and include devices that are nebulizers and inhalers. Oral delivery can be performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Direct injection techniques are particularly useful for administering a recombinant nucleic acid molecule to a cell or tissue that is accessible by surgery, and particularly, on or near the surface of the body. Administration of a composition locally within the area of a target cell refers to injecting the composition centimeters and preferably, millimeters from the target cell or tissue.

[0098] Various methods of administration and delivery vehicles disclosed herein have been shown to be effective for delivery of a nucleic acid molecule to a target cell or tissue, whereby the nucleic acid molecule transfected the cell and was expressed. In many studies, successful delivery and expression of a heterologous gene was achieved in preferred cell types and/or using preferred delivery vehicles and routes of administration of the present invention. Delivery of numerous nucleic acid sequences to a variety of target tissues has been accomplished by administration of viral vectors encoding the nucleic acid sequences. (e.g., see, of many examples, retroviral vector; Blaese et al., 1995, Science 270:475-480; Bordignon et al., 1995, Science 270:470-475), nasal administration (CFTR-adenovirus-associated vector), intracoronary administration (adenoviral vector and Hemagglutinating virus of Japan, see above), intravenous administration (adeno-associated viral vector; Koeberl et al., 1997, Proc Natl Acad Sci USA 94:1426-1431). Millecamps et al. reported the targeting of adenoviral vectors to neurons using neuron restrictive enhancer elements placed upstream of

the promoter for the transgene (phosphoglycerate promoter). Such vectors were administered to mice and rats intramuscularly and intracerebrally, respectively, resulting in successful neuronal-specific transfection and expression of the transgene *in vivo* (Millecamps et al., 1999, Nat. Biotechnol. 17:865-869). Bennett et al. reported the use of adeno-associated viral vector to deliver and express a gene by subretinal injection in the neural retina *in vivo* for greater than 1 year (Bennett, 1999, *ibid*.).

[0099] A suitable single dose of an inhibitory agent to administer to an animal is a dose that is capable of reducing or preventing at least one symptom of physiological damage due to TBI or SCI in an animal when administered one or more times over a suitable time period. A preferred single dose of an agent, including proteins, small molecules and antibodies, for use in the method described herein, comprises between about 0.01 microgram x -kilogram^{-1} and about 10 milligram x kilogram^{-1} body weight of an animal. A more preferred single dose of an agent comprises between about 1 microgram x kilogram^{-1} and about 10 milligram x kilogram^{-1} body weight of an animal. An even more preferred single dose of an agent comprises between about 5 microgram x kilogram^{-1} and about 7 milligram x kilogram^{-1} body weight of an animal. An even more preferred single dose of an agent comprises between about 10 microgram x kilogram^{-1} and about 5 milligram x kilogram^{-1} body weight of an animal. A particularly preferred single dose of an agent comprises between about 0.1 milligram x kilogram^{-1} and about 5 milligram x kilogram^{-1} body weight of an animal, if the an agent is delivered by aerosol. Another particularly preferred single dose of an agent comprises between about 0.1 microgram x kilogram^{-1} and about 10 microgram x kilogram^{-1}body weight of an animal, if the agent is delivered parenterally.

[0100] In one example, an appropriate single dose of a nucleic acid:liposome complex of the present invention is from about 0.1μg to about 100μg per kg body weight of the patient to which the complex is being administered. In another embodiment, an appropriate single dose is from about 1μg to about 10μg per kg body weight. In another embodiment, an appropriate single dose of nucleic acid:lipid complex is at least about 0.1μg of nucleic acid, more preferably at least about 1μg of nucleic acid, even more preferably at least about 10μg of nucleic acid, even more preferably at least about 50μg of nucleic acid, and even more preferably at least about 100μg of nucleic acid.

[0101] A preferred single dose of an antibody comprises between about 1 ng x kilogram^{-1} and about less than 1 mg x kilogram^{-1} body weight of an animal. A more preferred single dose of an antibody comprises between about 20 ng x kilogram^{-1} and about 600μg x kilogram^{-1} body weight of the animal. An even more preferred single dose of an antibody, particularly when the antibody formulation is delivered by nebulization, comprises between about 20 ng x kilogram^{-1} and about 600μg x kilogram^{-1} body weight of the animal, and more preferably, between about 20 ng x kilogram^{-1} and about 500μg x kilogram^{-1}, and more preferably, between about 20 ng x kilogram^{-1} and about 400μg x kilogram^{-1}, and more preferably, between about 20 ng x kilogram^{-1} and about 300μg x kilogram^{-1}, and more preferably, between about 20 ng x kilogram^{-1} and about 200μg x kilogram^{-1}, and more preferably, between about 20 ng x kilogram^{-1} and about 100μg x kilogram^{-1}, and more preferably, between about 20 ng x kilogram^{-1} and about 50μg x kilogram^{-1} body weight of the animal.

[0102] Another preferred single dose of an antibody, particularly when the antibody formulation is delivered by nebulization, comprises between about 200 ng x kilogram^{-1} and about 600μg x kilogram^{-1} body weight of the animal, and more preferably, between about 200 ng x kilogram^{-1} and about 500μg x kilogram^{-1}, and more preferably, between about 200 ng x kilogram^{-1} and about 400μg x kilogram^{-1}, and more preferably, between about 200 ng x kilogram^{-1} and about 300μg x kilogram^{-1}, and more preferably, between about 200 ng x kilogram^{-1} and about 200μg x kilogram^{-1}, and more preferably, between about 200 ng x kilogram^{-1} and about 100μg x kilogram^{-1}, and more preferably, between about 200 ng x kilogram^{-1} and about 50μg x kilogram^{-1} body weight of the animal.

[0103] Another preferred single dose of an antibody, particularly when the antibody formulation is delivered by direct inhalation from an inhaler, comprises between about 2 ng x kilogram^{-1} and about 100μg x kilogram^{-1} body weight of the animal, and more preferably, between about 2 ng x kilogram^{-1} and about 50μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 10μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 5μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 1μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 0.5μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 0.25μg x kilogram^{-1}, and more preferably, between about 2 ng x kilogram^{-1} and about 0.1μg x kilogram^{-1} body weight of the animal.

[0104] The antibody may be administered at a dose of less than about 500μg antibody per milliliter of formulation, and preferably, less than about 250μg antibody per milliliter of formulation, and more preferably, less than about 100μg antibody per milliliter of formulation, and more preferably, less than about 50μg antibody per milliliter of formulation, and more preferably, less than about 40μg antibody per milliliter of formulation, and more preferably, less than about 30μg antibody per milliliter of formulation, and more preferably, less than about 20μg antibody per milliliter of formulation, and more preferably, less than about 10μg antibody per milliliter of formulation, and even more preferably, between about 5 μg antibody and about 10μg antibody per milliliter of formulation.

[0105] According to the method described herein, an effective amount of an agent that inhibits physiological damage due to TBI or SCI to administer to an animal comprises an amount that is capable of reducing at least one symptom or indicator of physiological damage due to TBI or SCI, or is capable of enhancing recovery from TBI or SCI, without being toxic to the animal. An amount that is toxic to an animal comprises any amount that causes damage to the structure or

function of an animal (*i.e.,* poisonous).

[0106] In an animal that has experienced TBI or SCI, an effective amount of an agent to administer to an animal may be an amount that measurably reduces at least one symptom or indicator of physiological damage due to TBI or SCI in the animal as compared to prior to administration of the agent or as compared to in the absence of administration of the agent. An effective amount of an agent to administer to an animal may be an amount that measurably reduces at least one symptom or indicator of damage due to TBI or SCI in the animal as compared to a level of the symptom in a population of animals that have experienced substantially similar TBI or SCI wherein the agent was not administered. The agent is preferably capable of reducing at least one symptom or indicator of physiological damage due to TBI (*e.g.* brain damage) or SCI (*e.g.,* spinal cord damage) in an animal, even when the agent is administered after the onset of the physical symptoms of the damage. Most preferably, an effective amount of the agent is an amount that reduces the symptom(s) or indicator(s) of damage due to TBI or SCI to the point where the symptom(s) or indicator(s) is no longer detected in the patient. In one embodiment, an effective amount of the agent may be an amount that enhances the recovery of the patient from TBI or SCI, as measured by cessation or reversal of a symptom or indicator of physiological damage, or as measured by an improvement in a measurable or detectable biological score, value, or measure of neural and related functions in the patient.

[0107] A suitable dose of an agent of the present invention is a dose effective to inhibit the expression or biological activity of at least one protein in the alternative complement pathway as described herein (e.g., factor B, factor D or properdin), as compared to in the absence of the administration of the agent. Methods of measuring the expression or biological activity of a protein have been described above. In another embodiment, a suitable dose of an agent of the present invention is a dose that measurably inhibits the alternative complement pathway of the invention. Activation of complement and inhibition thereof can be measured using techniques/assays that are well-known in the art. For example, one can perform an *in vitro* analysis of C3 deposition on zymosan A particles as described in the examples. One can also test the ability of the agent to inhibit lysis of unsensitized erythrocytes by human serum. Extrapolation of *in vitro* results to *in vivo* dosages based on these assays is within the ability of those of skill in the art.

[0108] One of skill in the art will be able to determine that the number of doses of an agent to be administered to an animal is dependent upon the extent of the TBI or SCI and anticipated or observed physiological damage associated with the injury, as well as the response of an individual patient to the treatment. Methods to diagnose both TBI and SCI, including the severity of the conditions, are described above and are known in the art. In addition, the clinician will be able to determine the appropriate timing for delivery of the agent in a manner effective to reduce the symptom(s) associated with or resulting from TBI or SCI in the animal. Preferably, the agent is delivered within 48 hours, and more preferably 36 hours, and more preferably 24 hours, and more preferably within 12 hours, and more preferably within 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, or 1 hour, or even minutes after the event that caused the TBI or SCI. In one example, the agent is administered as soon as it is recognized (*i.e.,* immediately) by the patient, clinician, or other person that the patient has suffered TBI or SCI. In another embodiment, the agent is administered upon the first sign of development of a symptom of brain or neural damage that may be associated with TBI or SCI, and preferably, within at least 2 hours of the development of the symptom(s), and more preferably, within at least 1 hour, and more preferably within at least 30 minutes, and more preferably within at least 10 minutes, and more preferably within at least 5 minutes of development of the symptom(s). Symptoms of physiological damage associated with TBI or SCI and methods for measuring or detecting such symptoms have been described in detail above. Preferably, such administrations are given until signs of reduction of physiological damage or reduction of the symptoms of the potential for physiological damage appear, and then as needed until the symptoms are gone or arrested.

[0109] The method of the present invention can be used in any animal, and particularly, in any animal of the Vertebrate class, Mammalia (i.e., mammals), including, without limitation, primates, rodents, livestock and domestic pets. Preferred mammals to treat using the method of the present invention are humans.

[0110] The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

Examples

Example 1

[0111] The following example describes the therapeutic effect of administration of the complement inhibitor, Crry-Ig, on physiological functions after traumatic brain injury (TBI).

[0112] Crry-Ig is a fusion between complement receptor-related protein-y (Crry) and an immunoglobulin Fc molecule. Crry is a functional homologue of human decay-accelerating factor (CD55) and membrane-cofactor protein (CD46) and inhibits C3 complement convertase. Therefore, Crry is an inhibitor of both the classical and alternative complement pathways.

[0113] In a standardized model of closed head injury in mice (Chen et al., J. Neurotrauma 1996), the post-injury

systemic administration of 1 mg Crry-Ig, which corresponds to the therapeutic "time window of opportunity" due to a breached blood-brain-barrier from 1-24h after trauma in this model system, lead to a significantly improved neurological recovery after TBI within 24 h, as opposed to control mice injected with vehicle only (Fig. 6). In this experiment, the extent of posttraumatic neurological impairment was assessed by a standardized 10-point *Neurological Severity Score* (NSS) in a blinded fashion by two independent investigators. In addition, head-injured mice injected with 1 mg Crry-Ig i.p. one hour after trauma showed a significantly reduced weight loss compared to vehicle-injected controls (Fig. 7), indicating that the inflammation-induced posttraumatic catabolic state is protected in mice with complement inhibition by Crry-Ig.

[0114] These results demonstrate that inhibition of the complement pathway at the level of the C3 complement convertase inhibits physiological damage associated with TBI.

Example 2

[0115] The following example demonstrates that Factor B monoclonal antibody reduced brain damage associated with traumatic brain injury (TBI).

[0116] Preliminary titration studies revealed that *in vivo*, the intraperitoneal (i.p.) injection of 2 mg mAb1379 (factor B monoclonal antibody, or anti-fB) in C57BL/6 mice weighing 25-35 g resulted in complete inhibition of alternative pathway complement activity, lasting for 48 hours. Anti-fB inhibition studies after experimental closed head injury in C57BL/6 mice (Chen et al., J. Neurotrauma 1996) were performed using two experimental groups: one receiving 2 mg mAb1379, injected i.p. at t=lh, 24h, or 72h; and the second receiving vehicle medium alone, injected at identical time-points. The results revealed a significant neuroprotective effect of alternative pathway complement inhibition in the anti-fB (mAb1379) group, as compared to vehicle-injected control animals, based on a significantly decreased 10-point Neurological Severity Score (NSS) within 24 h after trauma (Table 3; Fig. 8). This result demonstrates that selective inhibition of the alternative complement pathway reduces physiological damage resulting from TBI.

Table 3

Neurological Severity Score (NSS)	**0 (best) -10 (worst)**	
	NSS 4h	***NSS 24h***
Trauma vehicle (n = 20)*	6	4
Trauma anti-factor B (n = 8)*	3.5	2.5
*median values		

Example 3

[0117] The following example shows that Factor B monoclonal antibody reduced brain damage associated with spinal cord injury (SCI).

[0118] Wild-type female C57BL/6 mice (Charles River, MD) and female fB-/- mice, 6-8-weeks old and 16-20g in weight (for all mice) were used in this study. Animals were provided water and food *ad libitum* and were housed in ventilated Plexiglas cages (four mouse/ per cage) on a 12/12-h light- dark cycle with a pathogen-free barrier facility and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals of the United States Department of Health and Human Services (National Institutes of Health, Bethesda, MD). Mice were acclimated for at least 1 week prior to experimentation.

[0119] Animal surgical and postoperative procedures were approved by the Committee on Animal Research at the Medical University of South Carolina. Surgical procedures were carried out under sterile conditions. Spinal cord contusions were performed using a weight drop device for the induction of spinal cord injury (SCI) in mice (Pannu et al. (2005) J. Neuroscience Research 79:340-350). Briefly, mice were anesthetized with an intraperitoneal (i.p.) injection of ketamine (75 mg/kg) and xylazine (16 mg/kg). The dorsal aspect of the back was shaved and scrubbed with iodinated solution. The body temperature was maintained throughout the surgery by a 37°C warming blanket placed under the animal. A midline incision was made in the skin from the T9 to T13 levels. Laminectomy was performed at the T12 level, leaving the dura intact. The spine was immobilized with a stereotactic device and injury was induced by dropping a weight of 5g from a height of 3cm (15g-cm force) onto the exposed dura. Sham-operated animals underwent laminectomy only. After injury, the muscles were closed in layers and the incision was sutured. The mice were kept on a heating pad. No pre- or postoperative prophylactic antibiotics or analgesics were used in order to avoid their possible interactions with the experimental therapy of SCI. Bladders were manually expressed twice daily until adequate spontaneous voiding returned.

[0120] All animals were included in the study of functional recovery. Hindlimb function of experimental mice was

assessed weekly by blinded observers using the hindlimb motor function score (Shuhei K J. of Neuropathology and Experimental Neurology (2004) 64-72). The scale ranged from 0 to 5, and scores were as follows: 0: No movement of the hindlimbs; 1: Barely perceptible movement of any hindlimb joints (hip, knee, or ankle); 2: Brisk movements at one or more hindlimb joints (hip, knee, or ankle) in one or both limbs but no co-ordination; 3: Alternate stepping and propulsive movements of hindlimbs but no weight bearing; 4: Weight bearing and can walk with some deficit; 5: Normal walking.

[0121] Fig. 9 shows the results of a study investigating the effect of the administration of factor B monoclonal antibody on functional recovery from spinal cord injury. In the murine model for SCI described above, C57BL/6 mice received two intravenous injections of 1mg/10g anti-factor B (mAb 1379) at 1 hour and 12 hours post injury (Group Number, n=8). Functional recovery was evaluated as described above once a day for 21 days post injury. Sham-treated mice, untreated mice with experimental SCI, and factor B (-/-) mice with experimental SCI were also evaluated over the time course. As shown in Fig. 9, factor B (-/-) mice and mice that received anti-factor B had a significantly ($p<0.01$) improved functional recovery score at each of the 21 day time points, as compared to untreated mice with experimental spinal cord injury. These results demonstrate that selective inhibition of the alternative complement pathway is sufficient to provide a significant therapeutic benefit in a model of SCI.

Example 4

[0122] The following example demonstrates that the alternative pathway of complement activation plays a crucial role in the pathophysiology of TBI.

Materials and Methods

[0123] *Factor B-/- mice.* The genetic knockout mice deficient in factor B (fB-/-) were previously characterized and shown to have a complete lack of a functional alternative complement pathway [58]. They were originally created with Sv129 embryonic stem cells and crossed with C57BL/6 mice prior to expansion of the colony at F1. They were then back-crossed for more than 10 generations against a pure C57BL/6 background and found to be grossly indistinguishable from C57BL/6 mice [34]. Knockout mice and wild-type littermates (fB+/+) were acclimatized several weeks before the experiments and kept isolated from external influences during the entire time course of the study. They were bred in a selective pathogen-free (SPF) environment and standardized conditions of temperature (21oC), humidity (60%), light and dark cycles (12:12h), with food and water provided ad libitum. Only male mice were used for this study in order to avoid a bias in gender with regard to levels of complement activity [59] and to susceptibility to brain injury which seems to be significantly influenced by female reproductive hormones [60,61]. All experiments were performed in compliance with the standards of the Federation of European Laboratory Animal Science Association (FELASA) and were approved by the institutional animal care committee (Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit, Berlin, Germany, No. G0099/03 and No. G0308/04).

[0124] *Brain injury model.* Experimental closed head injury was performed in knockout (fB-/-) mice and wild-type littermates (fB+/+) of the C57BL/6 strain (n=6 per group and time-point) using a standardized weight-drop device, as previously described [13,38,62-64]. In brief, after induction of isoflurane anesthesia, the skull was exposed by a midline longitudinal scalp incision. A 333 g weight was dropped on the fixed skull from a height of 2 cm, resulting in a focal blunt injury to the left hemisphere. After trauma, the mice received supporting oxygenation with 100% 02 until fully awake and were then brought back to their cages. At defined time-points (t=4h, 24h, and 7 days), mice were euthanized and brain hemispheres were extracted for analysis by immunohistochemistry, TUNEL histochemistry, and SDS-PAGE/Western blot analysis. In addition, serum samples were collected for determination of complement anaphylatoxin C5a levels by ELISA and Western blot analysis of Bcl-2 (see below).

[0125] Sham-operated mice were kept under identical conditions as the trauma group and underwent the same procedures (anesthesia and scalp incision) except that no head injury was applied.

[0126] *ELISA for mouse C5a.* For determination of complement anaphylatoxin C5a levels in serum samples of head-injured and normal C57BL/6 control mice, an ELISA developed in the laboratory of Dr. P.A. Ward (Ann Arbor, MI, USA) was used. In brief, ELISA plates (Immulon 4HBX, Thermo Labsystems, Milford, MA, USA) were coated with purified monoclonal anti-mouse C5a IgG (5 μg/ml, BD Pharmingen, San Diego, CA, USA). After blocking of non-specific binding sites with 1% milk (Roth, Karlsruhe, Germany) in PBS (Gibco-Invitrogen, Carlsbad, CA, USA) containing 0.05% TWEEN 20 (Sigma-Aldrich, St. Louis, MO, USA), the plate was coated with 100 ml serum diluted 1:20 (in 0.1% milk in PBS containing 0.05% TWEEN) and murine recombinant mouse C5a at defined concentrations for establishing the standard curve. After incubation and subsequent washing steps, biotinylated monoclonal anti-mouse C5a antibody was added at 500 ng/ml (BD Pharmingen) followed by washing steps and incubation with streptavidin-peroxidase at 400 ng/ml (Sigma). For colorimetric reaction, the substrate (0.4 mg/ml OPD with 0,4mg/ml urea hydrogen peroxide in 0.05M phosphate citrate buffer; Sigma) was added and the color reaction was stopped with 3M sulfuric acid. The absorbance was read at 490 nm ("SpectraMax 190" reader, Molecular Devices, Sunnyvale, CA, USA. All samples were analyzed in duplicate

wells and results were calculated from the means of duplicate sample analysis. The standard curve was linear from 50 ng/ml to 0.1 ng/ml, which represents the lower limit of detection of this assay.

[0127] *Western blot.* All mice used in this study were screened by Western blot analysis for the presence of factor B in serum, as an internal quality control. The protein levels of the mitochondrial anti-apoptotic mediator Bcl-2 and of the pro-apoptotic Fas receptor were determined in homogenized mouse brains and matched serum samples at 4h, 24h and 7d following head injury or sham operation in fB-/- and fB+/+ mice. The Western blot technique was previously described [32]. Briefly, mouse brains were extracted under anesthesia, separated into left and right hemispheres, and immediately homogenized in lysis buffer (Sigma) containing 100 mM TRIS-HCl (pH 7.5), 150 mM NaCl, 0.5% sodium dodecyl sulfate (SDS), 0.5% Nonidet P-40, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 5 mg/ml pepstatin, 1 mM phenyl-methyl-sulfonyl fluoride in deionized water, using an Ultra Turrax Homogenizer® (IKA Werke, Staufen, Germany). After 15 min centrifugation at 13,000 x g, the protein content of the supernatants was determined by commercially available colorimetric protein assay ("BCA Protein Assay", Pierce/Perbio Science, Bonn, Germany). A 60 μg sample of total protein was denatured in loading buffer and separated under reducing conditions on 12% SDS-polyacrylamide gels in parallel with a broad range prestained SDS-PAGE protein standard (Bio-Rad, Munich, Germany). Proteins were then transferred to Protran BA 83 nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) by electroblotting (Bio-Rad). The blots were blocked overnight and then incubated with either monoclonal anti-mouse Bcl-2 (Santa Cruz Biotechnology, Heidelberg, Germany), diluted 1:500, polyclonal rabbit anti-mouse Fas (clone A-20, Santa Cruz), diluted 1:200, polyclonal chicken anti-mouse anti-factor B, diluted 1:8,000 (kindly provided by Dr. Scott R. Barnum, University of Alabama at Birmingham, AL, USA) as primary antibodies, and with a monocloncal anti-β-actin antibody (clone AC-15, Sigma) diluted 1:10,000, as internal control for ascertaining equal loading of the bands. After incubation with peroxidase-labelled secondary antibodies (Dako, Hamburg, Germany, and Santa Cruz Biotechnology, Heidelberg, Germany), diluted 1:5,000, antibody binding was visualized by a non-radioactive chemiluminescence technique using a commercially available ECL® Western blotting kit (Amersham Pharmacia Biotech, Freiburg, Germany). Equal transfer of proteins to the blotting membrane was confirmed by ponceau red staining (Sigma).

[0128] *Immunohistochemistry.* For assessment of neuronal morphology, integrity, and apoptosis, extracted mouse brains were snap-frozen in liquid nitrogen, embedded in OCT compound (Sakura Finetek, Torrance, CA) and stored at -80°C until used for analysis. Six to eight-micrometer thick coronal tissue sections were cut with a cryostat at -20°C. For immunohistochemistry, slides were fixed in acetone and then analyzed by a standard biotin/avidin/peroxidase technique with DAB-tetrahydrochloride as chromogen (Vector, Burlingame, CA), as previously described [13,32]. The following primary antibodies were used as cell-markers: monoclonal anti-NeuN, at a titrated dilution of 1:2,000 (Chemicon, Hampshire, UK) for neurons; polyclonal rabbit anti-GFAP, 1:100 (Shandon Immunon, Pittsburgh, PA, USA) for astrocytes; monoclonal rat anti-CD11b, 1:100, (Accurate Chemical, Westbury, NY, USA) for microglia; polyclonal goat-anti CD144, 1:200 (Santa Cruz) for endothelial cells. Non-immunized IgG (Vector) was used as negative control at equal dilutions as the omitted specific antibody.

[0129] To determine the extent of intracerebral neuronal cell death, TUNEL histochemistry was performed using a "Fluorescein In Situ Cell Death Detection Kit" (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions, as previously described [38]. Briefly, slides were dried for 30 min followed by fixation in 10% formalin solution at RT. After washing in PBS (three times for 3 min), sections were incubated in ice-cold ethanol-acetic acid solution (2:1) for 5 min at -20°C. Thereafter, they were washed in PBS and incubated in a permeabilization solution with 3% Triton X-100 in PBS for 60 min at RT, then incubated with the TdT enzyme in a reaction buffer containing fluorescein-dUTP for 90 min at 37°C. Negative control was performed using only the reaction buffer without TdT enzyme. Positive controls were performed by digesting equal brain sections with DNase grade I solution (500U/ml; Roche) for 20min at RT and always kept separate from the other samples thereafter. After labelling, the sections were washed again in PBS and to visualize the unstained (TUNEL-negative) cells, the sections were covered with VectashieldO mounting medium for fluorescence with DAPI (Vector). All samples were evaluated immediately after staining using an Axioskop 40 fluorescence microscope (Zeiss, Germany) at 460 nm for DAPI and 520 nm for TUNEL fluorescence and analyzed by Alpha digi doc 1201 software (Alpha Innotech, San Leandro, CA, USA).

[0130] *Statistical analysis.* Statistical analysis was performed using commercially available software (SPSS 9.0 for Windows®). Differences in complement C5a levels in serum of fB-/- and fB+/+ mice were determined by the unpaired Student's t-test. A P-value < 0.05 was considered statistically significant.

Results

Complement activation is attenuated in brain-injured fB-/- mice.

[0131] Screening of serum samples from all fB-/- mice and wild-type littermates (fB+/+) used in the present study revealed that factor B was only detectable in serum of fB+/+ animals, but not in the fB-/- mice. These control experiments were performed to ascertain that the knockout mice are completely devoid of factor B in serum (data not shown).

[0132] Referring to Fig. 10, serum samples from brain-injured fB+/+ and fB-/- mice of the C57BL/6 strain (n=6 per group and time-point) and from normal C57BL/6 mice (control; n=4) were analyzed by ELISA specific for mouse C5a (data in Fig. 10 are shown as mean levels ±D; *P<0.05, fB+/+ vs. control and fB+/+ vs. fB-/- mice. TBI, traumatic brain injury). Experimental closed head injury in wild-type C57BL/6 mice resulted in a systemic activation of the complement cascade, as determined by significantly elevated serum levels of the complement activation product C5a at all time-points assessed from 4 hours to 7 days (P<0.05 vs. normal mouse serum, unpaired Student's t-test; Fig. 10). In contrast, anaphylatoxin C5a serum levels were dramatically reduced in fB-/- mice at all corresponding time-points after head trauma, down to baseline levels in normal mice (P<0.05 vs. brain-injured fB+/+ mice, unpaired Student's t-test; Fig. 10). These data imply that the alternative pathway is the source for complement activation after brain injury, a notion which has only previously been substantiated for diseases *outside* the CNS, such as rheumatoid arthritis, autoimmune nephritis, and ischemia/reperfusion injuries [33,37].

The lack offactor B leads to reduced neuronal cell death after head injury.

[0133] As previously described, neuronal cell death was observed in injured brains of wild-type C57BL/6 mice for up to 7 days after closed head injury [38]. Referring to Figs. 12-14, coronal cryosections of the left (injured) hemisphere of wild-type (fB+/+, panels A-E in each figure) and factor B knockout mice (fB-/-, panels F-J in each figure) were analyzed by immunohistochemistry with a specific antibody to the neuronal marker NeuN (A,B,F,G in each figure) or by TUNEL-histochemistry of adjacent sections (D,E,I,J in each figure). The overall cellular morphology of the TUNEL sections is revealed by DAPI nuclear stain (C,H in each figure). The panels B,E,G,J in each figure represent a 4-fold magnification of the respective panels A,D,F,I in each figure (Original magnifications: 100x (A,C,D,F,H,I), 400x (B,E,G,J)). An increase in TUNEL-positive cells was detected in the injured hemispheres of fB+/+ mice within 4 to 24 hours after trauma (Figs. 12 and 13, respectively), persisting for up to 7 days (Fig. 14). The nuclear staining with 4',6'-diamino-2-phenylindole (DAPI; panels C and H of Figs. 12-14) showed the cellular morphology in adjacent sections to those assessed by TUNEL histochemistry. Neurons were determined as the main TUNEL-positive cell-type by immunohistochemical staining of adjacent sections with the specific cell-marker NeuN (panels A, B, F and G of Figs. 12-14). In contrast, the staining of astrocytes (anti-GFAP), microglia (anti-CD11b), and endothelial cells (anti-CD144) revealed that these resident cells in the brain do not exhibit a relevant TUNEL-staining in the present model of head injury (data not shown). Furthermore, neurons were confirmed as the predominant TUNEL-positive cell-type by their typical cellular size and morphology (as opposed to glial cells) and to the typical neuronal layers of TUNEL-positive cells in the injured cortex. These findings corroborate previously published data on neuronal apoptosis in the current and other experimental TBI models as well as in head-injured patients [38-42]. In contrast to the extent of neuronal cell death in brain-injured fB+/+ mice, the fB-/- animals showed a clear reduction in TUNEL-positive neurons in injured brains from 4 hours to 7 days after trauma (Panels D, E, I and J of Figs. 12-14). These findings support the recently established concept of complement-dependent regulation of neuronal apoptosis [7,10,15,43] and promote the *in vivo* significance of the alternative (factor B-dependent) pathway of complement activation in regulating the extent of secondary neurodegeneration after TBI.

[0134] This is the first study, to the best of the present inventors knowledge, which investigated exclusively the role of the alternative pathway in contributing to neuropathology after brain injury. The fB-/- mice have previously been shown to be protected from experimental demyelination in an animal model of multiple sclerosis [44]. The studies by Nataf and colleagues support the present inventors' present findings in that the genetic deficiency of factor B, which provokes the complete lack of a functional alternative complement activation pathway, plays an essential role for neuroprotection in models of autoimmune and traumatic CNS injury.

Upregulation of Bcl-2 and downregulation of Fas in injured fB-/- brains

[0135] Posttraumatic neuronal apoptosis has been shown to be promoted by the Fas-mediated extrinsic pathway and by a suppression of the mitochondrial anti-apoptotic mediator Bcl-2 of the intrinsic pathway of apoptosis [45-51]. Referring to Fig. 11, homogenized brain tissue specimens from the injured hemispheres of sham-operated and head-injured fB+/+ and fB-/- mice were run out on SDS-PAGE, transferred to nitrocellulose membranes, and analyzed with specific monoclonal antibodies to Bcl-2 and detection by chemiluminescence assay (ECL® system, Amersham). The visualized 26 kDa band corresponding to mouse Bcl-2 is enhanced in the knockout mice at 24 hours, compared to head-injured wild-type littermates. Furthermore, a downregulation in Fas receptor staining intensity was obvious in brain-injured knockout mice at all time-points assessed, compared to fB+/+ mice (data not shown). The exemplary blot is representative of three independent experiments.

[0136] More particularly, by Western blot analysis, the inventors found a marked upregulation of protective Bcl-2 protein levels in brain homogenates of head-injured fB-/- mice at 24 hours after trauma, compared to fB+/+ littermates (Fig. 11). No apparent differences in Bcl-2 expression between knockout and wild-type mice were seen at other time-points after TBI (Fig. 11). With regard to the extrinsic pathway of apoptosis, a marked downregulation in Fas receptor expression

was seen within 4 hours to 7 days after TBI in fB-/- mice, compared to fB+/+ animals (Fig. 11). Although these data are not quantitative, the differences in staining intensity of the 26 kDa (Bcl-2; Fig. 11) and 48 kDa bands (Fas; data not shown) appear more intense in the brain-injured knockout mice than in the corresponding wild-type littermates at the above-mentioned time-points. These findings indicate an involvement of the alternative pathway of complement activation in regulating neuronal apoptosis after TBI by suppression of Bcl-2 and induction of Fas receptor expression in the injured brain. Both aspects are critical in the regulation of post-injury neuronal apoptosis, as previously determined by other investigators in different model systems [45-47]. An experimental study on a controlled cortical impact brain injury model demonstrated that the cortical lesion volume was significantly reduced in transgenic mice with over-expression of the Bcl-2 gene by 7 days after trauma, compared to wild-type littermates [52]. Thus, Bcl-2 was attributed an important role in the regulation of the mitochondrial (intrinsic) pathway of apoptosis after TBI [4,50,52,53].

[0137] The present inventors have previously shown that the pharmacological "pan"-inhibition of complement activation at the level of the C3 convertases by Crry-Ig, a murine recombinant chimeric fusion molecule, leads to enhanced intracerebral Bcl-2 gene and protein expression and to increased neuronal survival in the hippocampus of brain-injured mice [32]. In a model of murine autoimmune cerebritis, the blocking of complement activation by Crry-Ig resulted in a significant attenuation of neuronal apoptosis [15].

[0138] The data from the present study support the biological significance of the alternative pathway of complement activation in contributing to the neuropathological sequelae of TBI and provide the basis for future pharmacological studies with selective alternative pathway inhibitors, *e.g*. such as factor B antagonists [33,54].

[0139] In summary, the present data provide first evidence of a major role of the alternative pathway of complement activation in contributing to the overall extent of posttraumatic complement activation (C5a generation) and to secondary neuronal cell death after brain injury (TUNEL, Bcl-2, and Fas data). This is a new and provocative discovery, since all previously published studies on experimental complement inhibition in TBI models have focused on interfering with the complement cascade at the "common junction" level of C3 convertases [26,28-32] or further downstream in the cascade, e.g. by specific blocking of anaphylatoxin C5a or its receptor [30]. The hitherto underestimation of the pathophysiological role of the alternative complement pathway in the neuropathology of brain injury may be in part due to the historically established predominant role of the classical pathway in various neurological diseases [55,56]. However, the results from the present study indicate that these insights may not necessarily reflect the "true" *in vivo* significance of the alternative complement pathway in a complex multifactorial neuroinflammatory disease, such as in the setting of TBI [57]. The fact that elevated factor B levels are present in the intrathecal compartment of severely head-injured patients [36] further supports the claim herein that the pharmacological targeting of factor B is reasonable and predictable.

References for Example 4

[0140]

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[0141] While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

SEQUENCE LISTING

[0142]

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Ser Glu Glu Glu Lys Lys Leu Thr Arg Lys Glu Val Tyr Ile Lys Asn
    610                 615                 620

Gly Asp Lys Lys Gly Ser Cys Glu Arg Asp Ala Gln Tyr Ala Pro Gly
625                 630                 635                 640
```

Tyr Asp Lys Val Lys Asp Ile Ser Glu Val Val Thr Pro Arg Phe Leu
645 650 655

Cys Thr Gly Gly Val Ser Pro Tyr Ala Asp Pro Asn Thr Cys Arg Gly
660 665 670

Asp Ser Gly Gly Pro Leu Ile Val His Lys Arg Ser Arg Phe Ile Gln
675 680 685

Val Gly Val Ile Ser Trp Gly Val Val Asp Val Cys Lys Asn Gln Lys
690 695 700

Arg Gln Lys Gln Val Pro Ala His Ala Arg Asp Phe His Ile Asn Leu
705 710 715 720

Phe Gln Val Leu Pro Trp Leu Lys Glu Lys Leu Gln Asp Glu Asp Leu
725 730 735

Gly Phe Leu

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<211> 70
<212> PRT
<213> Homo sapiens

<400> 3

Ala Arg Pro Gln Gly Ser Cys Ser Leu Glu Gly Val Glu Ile Lys Gly
1 5 10 15

Gly Ser Phe Arg Leu Leu Gln Glu Gly Gln Ala Leu Glu Tyr Val Cys
20 25 30

Pro Ser Gly Phe Tyr Pro Tyr Pro Val Gln Thr Arg Thr Cys Arg Ser
35 40 45

Thr Gly Ser Trp Ser Thr Leu Lys Thr Gln Asp Gln Lys Thr Val Arg
50 55 60

Lys Ala Glu Cys Arg Ala
65 70

<210> 4
<211> 60
<212> PRT
<213> Homo sapiens

<400> 4

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Ile His Cys Pro Arg Pro His Asp Phe Glu Asn Gly Glu Tyr Trp Pro
1               5                  10                  15

Arg Ser Pro Tyr Tyr Asn Val Ser Asp Glu Ile Ser Phe His Cys Tyr
            20                  25                  30

Asp Gly Tyr Thr Leu Arg Gly Ser Ala Asn Arg Thr Cys Gln Val Asn
        35                  40                  45

Gly Arg Trp Ser Gly Gln Thr Ala Ile Cys Asp Asn
    50                  55                  60
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<210> 5
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<213> Homo sapiens

<400> 5

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Gly Tyr Cys Ser Asn Pro Gly Ile Pro Ile Gly Thr Arg Lys Val Gly
1               5                  10                  15

Ser Gln Tyr Arg Leu Glu Asp Ser Val Thr Tyr His Cys Ser Arg Gly
            20                  25                  30

Leu Thr Leu Arg Gly Ser Gln Arg Arg Thr Cys Gln Glu Gly Gly Ser
        35                  40                  45
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<400> 6

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Met Glu Ser Pro Gln Leu Cys Leu Val Leu Leu Val Leu Gly Phe Ser
1               5                  10                  15

Ser Gly Gly Val Ser Ala Thr Pro Val Leu Glu Ala Arg Pro Gln Val
            20                  25                  30

Ser Cys Ser Leu Glu Gly Val Glu Ile Lys Gly Gly Ser Phe Gln Leu
        35                  40                  45

Leu Gln Gly Gly Gln Ala Leu Glu Tyr Leu Cys Pro Ser Gly Phe Tyr
    50                  55                  60

Pro Tyr Pro Val Gln Thr Arg Thr Cys Arg Ser Thr Gly Ser Trp Ser
65                  70                  75                  80

Asp Leu Gln Thr Arg Asp Gln Lys Ile Val Gln Lys Ala Glu Cys Arg
                85                  90                  95
```

```
Ala Ile Arg Cys Pro Arg Pro Gln Asp Phe Glu Asn Gly Glu Phe Trp
            100                 105                 110

Pro Arg Ser Pro Phe Tyr Asn Leu Ser Asp Gln Ile Ser Phe Gln Cys
        115                 120                 125

Tyr Asp Gly Tyr Val Leu Arg Gly Ser Ala Asn Arg Thr Cys Gln Glu
    130                 135                 140

Asn Gly Arg Trp Asp Gly Gln Thr Ala Ile Cys Asp Asp Gly Ala Gly
145                 150                 155                 160

Tyr Cys Pro Asn Pro Gly Ile Pro Ile Gly Thr Arg Lys Val Gly Ser
                165                 170                 175

Gln Tyr Arg Leu Glu Asp Ile Val Thr Tyr His Cys Ser Arg Gly Leu
            180                 185                 190

Val Leu Arg Gly Ser Gln Lys Arg Lys Cys Gln Glu Gly Gly Ser Trp
        195                 200                 205

Ser Gly Thr Glu Pro Ser Cys Gln Asp Ser Phe Met Tyr Asp Ser Pro
    210                 215                 220

Gln Glu Val Ala Glu Ala Phe Leu Ser Ser Leu Thr Glu Thr Ile Glu
225                 230                 235                 240

Gly Ala Asp Ala Glu Asp Gly His Ser Pro Gly Glu Gln Gln Lys Arg
                245                 250                 255

Lys Ile Val Leu Asp Pro Ser Gly Ser Met Asn Ile Tyr Leu Val Leu
            260                 265                 270

Asp Gly Ser Asp Ser Ile Gly Ser Ser Asn Phe Thr Gly Ala Lys Arg
        275                 280                 285

Cys Leu Thr Asn Leu Ile Glu Lys Val Ala Ser Tyr Gly Val Arg Pro
    290                 295                 300

Arg Tyr Gly Leu Leu Thr Tyr Ala Thr Val Pro Lys Val Leu Val Arg
305                 310                 315                 320

Val Ser Asp Glu Arg Ser Ser Asp Ala Asp Trp Val Thr Glu Lys Leu
                325                 330                 335

Asn Gln Ile Ser Tyr Glu Asp His Lys Leu Lys Ser Gly Thr Asn Thr
            340                 345                 350
```

```
Lys Arg Ala Leu Gln Ala Val Tyr Ser Met Met Ser Trp Ala Gly Asp
        355                 360                 365

Ala Pro Pro Glu Gly Trp Asn Arg Thr Arg His Val Ile Ile Ile Met
    370                 375                 380

Thr Asp Gly Leu His Asn Met Gly Gly Asn Pro Val Thr Val Ile Gln
385                 390                 395                 400

Asp Ile Arg Ala Leu Leu Asp Ile Gly Arg Asp Pro Lys Asn Pro Arg
                405                 410                 415

Glu Asp Tyr Leu Asp Val Tyr Val Phe Gly Val Gly Pro Leu Val Asp
            420                 425                 430

Ser Val Asn Ile Asn Ala Leu Ala Ser Lys Lys Asp Asn Glu His His
        435                 440                 445

Val Phe Lys Val Lys Asp Met Glu Asp Leu Glu Asn Val Phe Tyr Gln
    450                 455                 460

Met Ile Asp Glu Thr Lys Ser Leu Ser Leu Cys Gly Met Val Trp Glu
465                 470                 475                 480

His Lys Lys Gly Asn Asp Tyr His Lys Gln Pro Trp Gln Ala Lys Ile
                485                 490                 495

Ser Val Thr Arg Pro Leu Lys Gly His Glu Thr Cys Met Gly Ala Val
            500                 505                 510

Val Ser Glu Tyr Phe Val Leu Thr Ala Ala His Cys Phe Met Val Asp
        515                 520                 525

Asp Gln Lys His Ser Ile Lys Val Ser Val Gly Gly Gln Arg Arg Asp
    530                 535                 540

Leu Glu Ile Glu Glu Val Leu Phe His Pro Lys Tyr Asn Ile Asn Gly
545                 550                 555                 560

Lys Lys Ala Glu Gly Ile Pro Glu Phe Tyr Asp Tyr Asp Val Ala Leu
                565                 570                 575

Val Lys Leu Lys Asn Lys Leu Lys Tyr Gly Gln Thr Leu Arg Pro Ile
            580                 585                 590

Cys Leu Pro Cys Thr Glu Gly Thr Thr Arg Ala Leu Arg Leu Pro Gln
        595                 600                 605
```

```
Thr Ala Thr Cys Lys Gln His Lys Glu Gln Leu Leu Pro Val Lys Asp
 610                 615                 620

Val Lys Ala Leu Phe Val Ser Glu Gln Gly Lys Ser Leu Thr Arg Lys
625                 630                 635                 640

Glu Val Tyr Ile Lys Asn Gly Asp Lys Lys Ala Ser Cys Glu Arg Asp
                645                 650                 655

Ala Thr Lys Ala Gln Gly Tyr Glu Lys Val Lys Asp Ala Ser Glu Val
            660                 665                 670

Val Thr Pro Arg Phe Leu Cys Thr Gly Gly Val Asp Pro Tyr Ala Asp
        675                 680                 685

Pro Asn Thr Cys Lys Gly Asp Ser Gly Gly Pro Leu Ile Val His Lys
 690                 695                 700

Arg Ser Arg Phe Ile Gln Val Gly Val Ile Ser Trp Gly Val Val Asp
705                 710                 715                 720

Val Cys Arg Asp Gln Arg Arg Gln Gln Leu Val Pro Ser Tyr Ala Arg
                725                 730                 735

Asp Phe His Ile Asn Leu Phe Gln Val Leu Pro Trp Leu Lys Asp Lys
            740                 745                 750

Leu Lys Asp Glu Asp Leu Gly Phe Leu
        755                 760
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<210> 7
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<212> PRT
<213> Homo sapiens

<400> 7

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Met His Ser Trp Glu Arg Leu Ala Val Leu Val Leu Leu Gly Ala Ala
1               5                  10                  15

Ala Cys Ala Ala Pro Pro Arg Gly Arg Ile Leu Gly Gly Arg Glu Ala
            20                  25                  30

Glu Ala His Ala Arg Pro Tyr Met Ala Ser Val Gln Leu Asn Gly Ala
        35                  40                  45

His Leu Cys Gly Gly Val Leu Val Ala Glu Gln Trp Val Leu Ser Ala
    50                  55                  60

Ala His Cys Leu Glu Asp Ala Ala Asp Gly Lys Val Gln Val Leu Leu

65                  70                  75                  80

Gly Ala His Ser Leu Ser Gln Pro Glu Pro Ser Lys Arg Leu Tyr Asp
                85                  90                  95

Val Leu Arg Ala Val Pro His Pro Asp Ser Gln Pro Asp Thr Ile Asp
            100                 105                 110

His Asp Leu Leu Leu Leu Gln Leu Ser Glu Lys Ala Thr Leu Gly Pro
        115                 120                 125

Ala Val Arg Pro Leu Pro Trp Gln Arg Val Asp Arg Asp Val Ala Pro
    130                 135                 140

Gly Thr Leu Cys Asp Val Ala Gly Trp Gly Ile Val Asn His Ala Gly
145                 150                 155                 160

Arg Arg Pro Asp Ser Leu Gln His Val Leu Leu Pro Val Leu Asp Arg
                165                 170                 175

Ala Thr Cys Asn Arg Arg Thr His His Asp Gly Ala Ile Thr Glu Arg
            180                 185                 190

Leu Met Cys Ala Glu Ser Asn Arg Arg Asp Ser Cys Lys Gly Asp Ser
        195                 200                 205

Gly Gly Pro Leu Val Cys Gly Gly Val Leu Glu Gly Val Val Thr Ser
    210                 215                 220

Gly Ser Arg Val Cys Gly Asn Arg Lys Lys Pro Gly Ile Tyr Thr Arg
225                 230                 235                 240

Val Ala Ser Tyr Ala Ala Trp Ile Asp Ser Val Leu Ala
                245                 250
```

<210> 8
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<212> PRT
<213> Homo sapiens

<400> 8

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Met Ile Thr Glu Gly Ala Gln Ala Pro Arg Leu Leu Leu Pro Pro Leu
1               5                  10                  15

Leu Leu Leu Leu Thr Leu Pro Ala Thr Gly Ser Asp Pro Val Leu Cys
            20                  25                  30

Phe Thr Gln Tyr Glu Glu Ser Ser Gly Lys Cys Lys Gly Leu Leu Gly
        35                  40                  45
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```
Gly Gly Val Ser Val Glu Asp Cys Cys Leu Asn Thr Ala Phe Ala Tyr
    50                  55                  60

Gln Lys Arg Ser Gly Gly Leu Cys Gln Pro Cys Arg Ser Pro Arg Trp
65                  70                  75                  80

Ser Leu Trp Ser Thr Trp Ala Pro Cys Ser Val Thr Cys Ser Glu Gly
                85                  90                  95

Ser Gln Leu Arg Tyr Arg Arg Cys Val Gly Trp Asn Gly Gln Cys Ser
            100                 105                 110

Gly Lys Val Ala Pro Gly Thr Leu Glu Trp Gln Leu Gln Ala Cys Glu
        115                 120                 125

Asp Gln Gln Cys Cys Pro Glu Met Gly Gly Trp Ser Gly Trp Gly Pro
    130                 135                 140

Trp Glu Pro Cys Ser Val Thr Cys Ser Lys Gly Thr Arg Thr Arg Arg
145                 150                 155                 160

Arg Ala Cys Asn His Pro Ala Pro Lys Cys Gly Gly His Cys Pro Gly
                165                 170                 175

Gln Ala Gln Glu Ser Glu Ala Cys Asp Thr Gln Gln Val Cys Pro Thr
            180                 185                 190

His Gly Ala Trp Ala Thr Trp Gly Pro Trp Thr Pro Cys Ser Ala Ser
        195                 200                 205

Cys His Gly Gly Pro His Glu Pro Lys Glu Thr Arg Ser Arg Lys Cys
    210                 215                 220

Ser Ala Pro Glu Pro Ser Gln Lys Pro Pro Gly Lys Pro Cys Pro Gly
225                 230                 235                 240

Leu Ala Tyr Glu Gln Arg Arg Cys Thr Gly Leu Pro Pro Cys Pro Val
                245                 250                 255

Ala Gly Gly Trp Gly Pro Trp Gly Pro Val Ser Pro Cys Pro Val Thr
            260                 265                 270

Cys Gly Leu Gly Gln Thr Met Glu Gln Arg Thr Cys Asn His Pro Val
        275                 280                 285

Pro Gln His Gly Gly Pro Phe Cys Ala Gly Asp Ala Thr Arg Thr His
    290                 295                 300
```

```
Ile Cys Asn Thr Ala Val Pro Cys Pro Val Asp Gly Glu Trp Asp Ser
305                 310                 315                 320

Trp Gly Glu Trp Ser Pro Cys Ile Arg Arg Asn Met Lys Ser Ile Ser
                325                 330                 335

Cys Gln Glu Ile Pro Gly Gln Gln Ser Arg Gly Arg Thr Cys Arg Gly
            340                 345                 350

Arg Lys Phe Asp Gly His Arg Cys Ala Gly Gln Gln Gln Asp Ile Arg
        355                 360                 365

His Cys Tyr Ser Ile Gln His Cys Pro Leu Lys Gly Ser Trp Ser Glu
    370                 375                 380

Trp Ser Thr Trp Gly Leu Cys Met Pro Pro Cys Gly Pro Asn Pro Thr
385                 390                 395                 400

Arg Ala Arg Gln Arg Leu Cys Thr Pro Leu Leu Pro Lys Tyr Pro Pro
                405                 410                 415

Thr Val Ser Met Val Glu Gly Gln Gly Glu Lys Asn Val Thr Phe Trp
            420                 425                 430

Gly Arg Pro Leu Pro Arg Cys Glu Glu Leu Gln Gly Gln Lys Leu Val
        435                 440                 445

Val Glu Glu Lys Arg Pro Cys Leu His Val Pro Ala Cys Lys Asp Pro
    450                 455                 460

Glu Glu Glu Glu Leu
465
```

Claims

1. An antibody or antigen-binding fragment thereof for use in the reduction or prevention of at least one symptom of physiological damage resulting from traumatic brain injury (TBI) or spinal cord injury (SCI) in an animal or to enhance recovery from TBI or SCI in the animal, wherein the antibody or antigen-binding fragment thereof selectively binds to and inhibits the activity of factor B, factor D, or properdin.

2. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof selectively binds to factor B within the third short consensus repeat (SCR) domain and prevents formation of a C3bBb complex.

3. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof binds to factor B and prevents or inhibits cleavage of factor B by factor D.

4. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof binds to the third short consensus repeat (SCR) domain of human factor B.

5. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-

binding fragment thereof selectively binds to an epitope in the third SCR domain of factor B selected from the group consisting of:

a) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from position Tyr139 to position Ser185;
b) an epitope of B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from position Tyr139 to position Ser141;
c) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO:2) comprising from position Glu182 to position Ser185;
d) an epitope of factor B that includes at least a portion of human factor B (SEQ ID NO: 2) comprising any one or more of the following positions: Tyr139, Cys140, Ser141, Glu182, Gly184, or Ser185;
e) an epitope in the third SCR domain of factor B (SEQ ID NO:2) comprising one or more of the following amino acid positions: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192;
f) an epitope in the third SCR domain of factor B (SEQ ID NO:2) comprising the following amino acid positions: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192;
g) an epitope in the third SCR domain of factor B (SEQ ID NO:2) consisting of the following amino acid positions: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, and Ser192; and
h) a non-linear epitope within the three-dimensional structure of a portion of the third SCR domain of factor B, wherein the portion is defined by at least amino acid positions Ala137-Ser192 of SEQ ID NO:2.

6. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof selectively binds to factor B-from multiple mammalian species and prevents formation of a C3bBb complex.

7. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof selectively binds to factor B from human and an animal selected from the group consisting of: non-human primate, mouse, rat, pig, horse and rabbit.

8. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody is of a non-complement activating isotype or subclass.

9. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of: a monoclonal antibody or antigen-binding fragment thereof, a humanized antibody or antigen-binding fragment thereof, a bispecific antibody or antigen-binding fragment thereof, a monovalent antibody or antigen-binding fragment thereof, and a chimeric antibody or antigen-binding fragment thereof.

10. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antigen-binding fragment is an Fab.

11. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof is: a) the monoclonal antibody 1379 (produced by ATCC Deposit No. PTA-6230) or an antigen-binding fragment thereof; or b) a monoclonal antibody or antigen-binding fragment thereof binding to the same epitope on factor B as mAb 1379 does, preferably wherein,
the antibody or antigen-binding fragment thereof is a humanized, or chimeric version of mAb 1379 or an antigen-binding fragment thereof.

12. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof is administered intravenously or to the brain of the animal experiencing TBI or is administered intravenously or to the spinal cord or epidural space of the spinal cord of the animal experiencing SCI.

13. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the antibody or antigen-binding fragment thereof measurably reduces at least one symptom of physiological damage resulting from TBI or SCI in the animal as compared to in the absence of administration of the antibody or antigen-binding fragment thereof.

14. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the animal has experienced TBI, and wherein the antibody or antigen-binding fragment thereof maintains a cerebral perfusion pressure (CPP) of above 70-80 mmHg, or lowers intracranial pressure (ICP).

15. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the animal has experienced SCI, and wherein the antibody or antigen-binding fragment thereof reduces swelling in the spinal cord.

16. The antibody or antigen-binding fragment thereof for use according to any one of claims 1-15, wherein the antibody or antigen-binding fragment thereof is administered in a pharmaceutically acceptable carrier.

17. The antibody or antigen-binding fragment thereof for use according to claim 16, wherein the pharmaceutically acceptable carrier is: a) a compound or composition that is capable of crossing the blood-brain barrier or b) an injectable excipient.

18. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein when the animal has experienced TBI, and wherein the antibody or antigen-binding fragment thereof is for use in conjunction with another compound for use in the treatment of a symptom of TBI selected from the group consisting of: a physical impairment, a cognitive impairment, and a psychosocial-behavioral-emotional impairment.

19. The antibody or antigen-binding fragment thereof for use according to claim 18, wherein the compound is selected from the group consisting of: an osmotic drug, a sedative, an analgesic, a muscle relaxant, and a barbiturate.

20. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein when the animal has experienced SCI, and wherein the antibody or antigen-binding fragment thereof is for use in conjunction with a steroid.

21. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein when the animal has experienced TBI, and wherein the antibody or antigen-binding fragment thereof is for use in conjunction with a protocol selected from the group consisting of: reduction of mass lesions by surgical evacuation of intracranial hematomas; reduction of brain swelling with osmotic drugs; therapeutic drainage of cerebrospinal fluid (CSF) through intraventricular catheters; computerized tomography (CT) scans; sedation; analgesia; muscle relaxation; moderate hyperventilation; moderate hypothermia; and barbiturate coma.

22. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the animal has experienced SCI, and wherein the antibody or antigen-binding fragment thereof is for use in conjunction with a protocol selected from the group consisting of: administration of steroids; immobilization of the spine; decompression surgery; surgery to stabilize the vertebrae; surgery to realign the vertebrae; and traction.

23. The antibody or antigen-binding fragment thereof for use according to any one of claims 1-23, wherein the animal is a mammal, preferably wherein the animal is a human.

24. The antibody or antigen-binding fragment thereof for use according to claim 1, wherein the symptom of TBI is cerebral vasospasms, cardiovascular depression, hypotension, hypoxemia, hypercarbia, hypoglycemia, posttraumatic neurological impairment, and neuronal cell death; and/or wherein the symptom of SCI is spinal cord swelling, motor function deficit, or deficit in walking ability.

25. A composition for use in the reduction or prevention of a symptom of traumatic brain injury (TBI) or spinal cord injury (SCI) in an animal or to enhance recovery from TBI or SCI in an animal, wherein the composition comprises:

    a) a first agent selected from the group consisting of: an isolated antibody or antigen-binding fragment thereof that selectively binds to and inhibits the activity of factor B, factor D, or properdin; and
    b) a second agent for use in the treatment of a symptom of TBI or SCI.

26. The composition for use according to claim 25, wherein the isolated antibody or antigen-binding fragment thereof binds to factor B within the third short consensus repeat (SCR) domain and inhibits or prevents formation of a C3bBb complex.

27. The composition for use according to claim 25 or 26, wherein the antibody or antigen-binding fragment thereof is: a) the monoclonal antibody 1379 (produced by ATCC Deposit No. PTA-6230) or an antigen-binding fragment thereof; or b) a monoclonal antibody or antigen-binding fragment thereof binding to the same epitope on factor B as mAb 1379 does, preferably wherein the antibody or antigen-binding fragment thereof is a humanized, or chimeric version of mAb 1379 or an antigen-binding fragment thereof.

28. The composition for use according to claim 25, wherein the second agent is a compound for treating a symptom of TBI selected from the group consisting of: a physical impairment, a cognitive impairment, and a psychosocial-behavioral-emotional impairment.

29. The composition for use according to claim 25, wherein the second agent for treating TBI is selected from the group consisting of: an osmotic drug, a sedative, an analgesic, a muscle relaxant, and a barbiturate.

30. The composition for use according to claim 25, wherein the second agent for treating SCI is a steroid.

31. The composition for use according to claim 25, wherein the symptom of TBI is a physical impairment, a cognitive impairment, a psychosocial-behavioral-emotional impairment, or neuronal cell death, and/or wherein the symptom of SCI is spinal cord swelling.

Patentansprüche

1. Ein Antikörper oder ein antigenbindendes Fragment davon zur Verwendung bei der Verminderung oder Verhinderung von mindestens einem Symptom einer physiologischen Störung als Ergebnis eines Schädel-Hirn-Traumas (SHT) oder einer Rückenmarksverletzung (SCI) in einem Tier oder zum Verbessern der Erholung von SHT oder SCI in dem Tier, wobei der Antikörper oder das antigenbindende Fragment davon selektiv an Faktor B, Faktor D oder Properdin bindet und die Aktivität davon hemmt.

2. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon selektiv an Faktor B innerhalb der dritten SCR-Domäne (SCR = Short Consensus Repeat, kurze übereinstimmende Wiederholung) bindet und die Bildung eines C3bBb-Komplexes verhindert.

3. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon an Faktor B bindet und die Spaltung von Faktor B durch Faktor D verhindert oder hemmt.

4. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon an die dritte SCR-Domäne des menschlichen Faktors B bindet.

5. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon selektiv an ein Epitop in der dritten SCR-Domäne von Faktor B, ausgewählt aus der Gruppe, bestehend aus Folgendem, bindet:

   a) einem Epitop von Faktor B, umfassend mindestens einen Teil des menschlichen Faktors B (SEQ ID NO: 2), beinhaltend von Position Tyr139 bis Position Ser185;
   b) einem Epitop von B, umfassend mindestens einen Teil des menschlichen Faktors B (SEQ ID NO: 2), beinhaltend von Position Tyr139 bis Position Ser141;
   c) einem Epitop von Faktor B, umfassend mindestens einen Teil des menschlichen Faktors B (SEQ ID NO: 2), beinhaltend von Position Glu182 bis Position Ser185;
   d) einem Epitop von Faktor B, umfassend mindestens einen Teil des menschlichen Faktors B (SEQ ID NO: 2), beinhaltend eine beliebige oder mehrere der folgenden Positionen: Tyr139, Cys140, Ser141, Glu182, Gly184 oder Ser185;
   e) einem Epitop in der dritten SCR-Domäne von Faktor B (SEQ ID NO: 2), beinhaltend eine oder mehrere der folgenden Aminosäurepositionen: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190 und Ser192;
   f) einem Epitop in der dritten SCR-Domäne von Faktor B (SEQ ID NO: 2), beinhaltend die folgenden Aminosäurepositionen: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190 und Ser192;
   g) einem Epitop in der dritten SCR-Domäne von Faktor B (SEQ ID NO: 2), bestehend aus den folgenden Aminosäurepositionen: Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190 und Ser192; und
   h) einem nicht linearen Epitop innerhalb der dreidimensionalen Struktur eines Teils der dritten SCR-Domäne von Faktor B, wobei der Teil durch mindestens die Aminosäurepositionen Ala137-Ser192 von SEQ ID NO: 2 definiert ist.

6. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon selektiv an Faktor B von mehreren Säugetierarten bindet und die Bildung

eines C3bBb-Komplexes verhindert.

7. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon selektiv an Faktor B von einem Menschen und einem Tier, ausgewählt aus der Gruppe, bestehend aus Folgendem, bindet: nichtmenschlichem Primaten, Maus, Ratte, Schwein, Pferd und Hase.

8. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper von einem nicht komplementaktivierenden Isotyp oder einer solchen Unterklasse ist.

9. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon aus der Gruppe ausgewählt ist, die aus Folgendem besteht: einem monoklonalen Antikörper oder antigenbindenden Fragment davon, einem vermenschlichten Antikörper oder antigenbindenden Fragment davon, einem bispezifischen Antikörper oder antigenbindenden Fragment davon, einem monovalenten Antikörper oder antigenbindenden Fragment davon und einem chimären Antikörper oder antigenbindenden Fragment davon.

10. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei das antigenbindende Fragment ein Fab ist.

11. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon Folgendes ist: a) der monoklonale Antikörper 1379 (produziert durch ATCC-Hinterlegung Nr. PTA-6230) oder ein antigenbindendes Fragment davon; oder b) ein monoklonaler Antikörper oder ein antigenbindendes Fragment davon, der/das an dasselbe Epitop an Faktor B wie mAb 1379 bindet, wobei vorzugweise
der Antikörper oder das antigenbindende Fragment davon eine vermenschlichte oder chimäre Version von mAb 1379 oder ein antigenbindendes Fragment davon ist.

12. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon intravenös oder in das Hirn des an SHT leidenden Tiers verabreicht wird, oder intravenös oder in das Rückenmark oder den Epiduralraum des Rückenmarks des an SCI leidenden Tiers verabreicht wird.

13. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei der Antikörper oder das antigenbindende Fragment davon mindestens ein Symptom einer physiologischen Störung als Ergebnis von SHT oder SCI in dem Tier im Vergleich zum Ausbleiben der Verabreichung des Antikörpers oder antigenbindenden Fragments davon messbar vermindert.

14. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei das Tier an SHT leidet, und wobei der Antikörper oder das antigenbindende Fragment davon einen zerebralen Perfusionsdruck (CPP) von über 70-80 mmHg aufrechterhält oder intrakraniellen Druck (ICP) senkt.

15. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei das Tier an SCI leidet, und wobei der Antikörper oder das antigenbindende Fragment davon Schwellung in dem Rückenmark vermindert.

16. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß einem der Ansprüche 1-15, wobei der Antikörper oder das antigenbindende Fragment davon in einem pharmazeutisch akzeptablen Träger verabreicht wird.

17. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 16, wobei der pharmazeutisch akzeptable Träger Folgendes ist: a) eine Verbindung oder Zusammensetzung, die in der Lage ist, die Blut-Hirn-Schranke zu passieren, oder b) ein injizierbarer Hilfsstoff.

18. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei, wenn das Tier an SHT leidet, und wobei der Antikörper oder das antigenbindende Fragment davon zur Verwendung zusammen mit einer weiteren Verbindung zur Verwendung bei der Behandlung eines SHT-Symptoms ist, ausgewählt aus der Gruppe, bestehend aus Folgendem: einer körperlichen Schwäche, einer kognitiven Schwäche und einer psycho-

sozialen/Verhaltens-/emotionalen Schwäche.

19. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 18, wobei die Verbindung aus der Gruppe ausgewählt ist, die aus Folgendem besteht:

einem osmotischen Arzneimittel, einem Sedativum, einem Analgetikum, einem Muskelrelaxans und einem Barbiturat.

20. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei, wenn das Tier an SCI leidet, und wobei der Antikörper oder das antigenbindende Fragment davon zur Verwendung zusammen mit einem Steroid ist.

21. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei, wenn das Tier an SHT leidet, und wobei der Antikörper oder das antigenbindende Fragment davon zur Verwendung zusammen mit einem Plan ist, ausgewählt aus der Gruppe, bestehend aus Folgendem: Verminderung von großflächigen Läsionen durch chirurgische Evakuierung intrakranieller Hämatome; Verminderung von Hirnschwellung mit osmotischen Arzneimitteln; therapeutischem Abfluss von Liquor (CSF) durch Ventrikelkatheter; rechnergestützten Tomographie-Scans (CT-Scans); Sedierung; Analgesie; Muskelrelaxation; moderater Hyperventilation; moderater Hypothermie; und Barbituratkoma.

22. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei das Tier an SCI leidet, und wobei der Antikörper oder das antigenbindende Fragment davon zur Verwendung zusammen mit einem Plan ist, ausgewählt aus der Gruppe, bestehend aus Folgendem: Verabreichung von Steroiden; Immobilisierung der Wirbelsäule; Dekompressionsoperation; Operation zur Stabilisierung der Wirbel; Operation zum Neuausrichten der Wirbel; und Traktion.

23. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß einem der Ansprüche 1-23, wobei das Tier ein Säugetier ist, wobei das Tier vorzugweise ein Mensch ist.

24. Antikörper oder antigenbindendes Fragment davon zur Verwendung gemäß Anspruch 1, wobei das SHT-Symptom zerebrale Vasospasmen, kardiovaskuläre Depression, Hypotonie, Hypoxämie, Hyperkapnie, Hypoglykämie, posttraumatische neurologische Schwäche und Absterben neuronaler Zellen ist; und/oder wobei das SCI-Symptom Rückenmarkschwellung, Motorikdefizit oder Defizit beim Gehvermögen ist.

25. Eine Zusammensetzung zur Verwendung bei der Verminderung oder Verhinderung eines Symptoms eines Schädel-Hirn-Traumas (SHT) oder einer Rückenmarksverletzung (SCI) in einem Tier oder zur Verbesserung der Erholung von SHT oder SCI in einem Tier, wobei die Zusammensetzung Folgendes beinhaltet:

a) ein erstes Mittel, ausgewählt aus der Gruppe, bestehend aus Folgendem: einem isolierten Antikörper oder antigenbindenden Fragment davon, der/das selektiv an Faktor B, Faktor D oder Properdin bindet und deren Aktivität hemmt; und
b) ein zweites Mittel zur Verwendung bei der Behandlung eines SHT- oder SCI-Symptoms.

26. Zusammensetzung zur Verwendung gemäß Anspruch 25, wobei der isolierte Antikörper oder das antigenbindende Fragment davon an Faktor B innerhalb der dritten SCR-Domäne bindet und die Bildung eines C3bBb-Komplexes hemmt oder verhindert.

27. Zusammensetzung zur Verwendung gemäß Anspruch 25 oder 26, wobei der Antikörper oder das antigenbindende Fragment davon Folgendes ist: a) der monoklonale Antikörper 1379 (produziert durch ATCC-Hinterlegung Nr. PTA-6230) oder ein antigenbindendes Fragment davon; oder b) ein monoklonaler Antikörper oder ein antigenbindendes Fragment davon, der/das an dasselbe Epitop an Faktor B wie mAb 1379 bindet, wobei vorzugweise der Antikörper oder das antigenbindende Fragment davon eine vermenschlichte oder chimäre Version von mAb 1379 oder ein antigenbindendes Fragment davon ist.

28. Zusammensetzung zur Verwendung gemäß Anspruch 25, wobei das zweite Mittel eine Verbindung zum Behandeln eines SHT-Symptoms ist, ausgewählt aus der Gruppe, bestehend aus Folgendem: einer körperlichen Schwäche, einer kognitiven Schwäche und einer psychosozialen/Verhaltens-/emotionalen Schwäche.

29. Zusammensetzung zur Verwendung gemäß Anspruch 25, wobei das zweite Mittel zum Behandeln von SHT aus der Gruppe ausgewählt ist, die aus Folgendem besteht: einem osmotischen Arzneimittel, einem Sedativum, einem Analgetikum, einem Muskelrelaxans und einem Barbiturat.

30. Zusammensetzung zur Verwendung gemäß Anspruch 25, wobei das zweite Mittel zum Behandeln von SCI ein Steroid ist.

31. Zusammensetzung zur Verwendung gemäß Anspruch 25, wobei das SHT-Symptom eine körperliche Schwäche, eine kognitive Schwäche, eine psychosoziale/Verhaltens-/emotionale Schwäche oder Absterben neuronaler Zellen ist und/oder wobei das SCI-Symptom Rückenmarkschwellung ist.

Revendications

1. Un anticorps ou un fragment de celui-ci se liant à un antigène pour une utilisation dans la réduction ou la prévention d'au moins un symptôme de dommage physiologique résultant d'une lésion traumatique cérébrale (LTC) ou d'une lésion à la moelle épinière (LME) chez un animal ou pour favoriser la récupération après une LTC ou une LME chez l'animal, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie de façon sélective au facteur B, au facteur D, ou à la properdine et inhibe leur activité.

2. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie de façon sélective au facteur B au sein du troisième domaine de la séquence consensuelle répétée (SCR) et empêche la formation d'un complexe C3bBb.

3. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie au facteur B et empêche ou inhibe le clivage du facteur B par le facteur D.

4. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie au troisième domaine de la séquence consensuelle répétée (SCR) du facteur humain B.

5. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie de façon sélective à un épitope dans le troisième domaine SCR du facteur B sélectionné dans le groupe consistant en :

   a) un épitope du facteur B qui inclut au moins une portion du facteur B humain (SEQ ID No : 2) comprenant de la position Tyr139 à la position Ser185 ;
   b) un épitope de B qui inclut au moins une portion du facteur B humain (SEQ ID No : 2) comprenant de la position Tyr139 à la position Ser141 ;
   c) un épitope du facteur B qui inclut au moins une portion du facteur B humain (SEQ ID No : 2) comprenant de la position Glu182 à la position Ser185 ;
   d) un épitope du facteur B qui inclut au moins une portion du facteur B humain (SEQ ID No : 2) comprenant n'importe laquelle ou lesquelles des positions suivantes : Tyr139, Cys140, Ser141, Glu 182, Gly184, ou Ser185 ;
   e) un épitope dans le troisième domaine SCR du facteur B (SEQ ID No : 2) comprenant une ou plusieurs des positions d'acides aminés suivantes : Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, et Ser192 ;
   f) un épitope dans le troisième domaine SCR du facteur B (SEQ ID No : 2) comprenant les positions d'acides aminés suivantes : Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, et Ser192 ;
   g) un épitope dans le troisième domaine SCR du facteur B (SEQ ID No : 2) consistant en les positions d'acides aminés suivantes : Ala137, Tyr139, Ser141, Glu182, Ser185, Thr189, Glu190, et Ser192 ; et
   h) un épitope non linéaire au sein de la structure tridimensionnelle d'une portion du troisième domaine SCR du facteur B, la portion étant définie par au moins les positions d'acides aminés Ala137 à Ser192 de SEQ ID No : 2.

6. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène se lie de façon sélective au facteur B d'espèces mammaliennes multiples et empêche la formation d'un complexe C3bBb.

7. L'anticorps ou le fragment de liaison à un antigène de celui-ci pour une utilisation selon la revendication 1, lequel

anticorps ou fragment de celui-ci se liant à un antigène se lie de façon sélective au facteur B d'un humain et d'un animal sélectionné dans le groupe consistant en : un primate non humain, une souris, un rat, un cochon, un cheval et un lapin.

8. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps est d'un isotype ou d'une sous-classe n'activant pas le complément.

9. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène est sélectionné dans le groupe consistant en : un anticorps monoclonal ou un fragment de celui-ci se liant à un antigène, un anticorps humanisé ou un fragment de celui-ci se liant à un antigène, un anticorps bispécifique ou un fragment de celui-ci se liant à un antigène, un anticorps monovalent ou un fragment de celui-ci se liant à un antigène, et un anticorps chimérique ou un fragment de celui-ci se liant à un antigène.

10. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel fragment se liant à un antigène est un Fab.

11. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène est : a) l'anticorps monoclonal 1379 (produit par ATCC Dépôt N° PTA-6230) ou un fragment de celui-ci se liant à un antigène ; ou b) un anticorps monoclonal ou un fragment de celui-ci se liant à un antigène qui se lie au même épitope sur le facteur B que mAb 1379, de préférence où, l'anticorps ou le fragment de celui-ci se liant à un antigène est une version humanisée, ou chimérique de mAb 1379 ou un fragment de celui-ci se liant à un antigène.

12. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène est administré de façon intraveineuse ou dans le cerveau de l'animal souffrant de LTC ou est administré de façon intraveineuse ou dans la moelle épinière ou l'espace épidural de la moelle épinière de l'animal souffrant de LME.

13. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, lequel anticorps ou fragment de celui-ci se liant à un antigène réduit de façon mesurable au moins un symptôme de dommage physiologique résultant de LTC ou de LME chez l'animal par comparaison à lorsqu'il y a absence d'administration de l'anticorps ou du fragment de celui-ci se liant à un antigène.

14. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où l'animal a souffert de LTC, et lequel anticorps ou fragment de celui-ci se liant à un antigène maintient une pression de perfusion cérébrale (PPC) au-dessus de 70 à 80 mmHg, ou abaisse la pression intracrânienne (PIC).

15. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où l'animal a souffert de LME, et lequel anticorps ou fragment de celui-ci se liant à un antigène réduit une tuméfaction dans la moelle épinière.

16. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon n'importe laquelle des revendications 1 à 15, lequel anticorps ou fragment de celui-ci se liant à un antigène est administré dans un support acceptable d'un point de vue pharmaceutique.

17. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 16, où le support acceptable d'un point de vue pharmaceutique est : a) un composé ou une composition qui est capable de traverser la barrière sang-cerveau ou b) un excipient injectable.

18. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où lorsque l'animal a souffert de LTC, et lequel anticorps ou fragment de celui-ci se liant à un antigène est pour une utilisation en conjonction avec un autre composé pour une utilisation dans le traitement d'un symptôme de LTC sélectionné dans le groupe consistant en : une déficience physique, une déficience cognitive, et une déficience psychosociale-comportementale-émotionnelle.

19. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 18, où le composé est sélectionné dans le groupe consistant en : une drogue osmotique, un sédatif, un analgésique, un

relaxant musculaire, et un barbiturique.

20. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où lorsque l'animal a souffert de LME, et lequel anticorps ou fragment de celui-ci se liant à un antigène est pour une utilisation en conjonction avec un stéroïde.

21. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où lorsque l'animal a souffert de LTC, et lequel anticorps ou fragment de celui-ci se liant à un antigène est pour une utilisation en conjonction avec un protocole sélectionné dans le groupe consistant en : une réduction des lésions massives par évacuation chirurgicale d'hématomes intracrâniens ; une réduction de la tuméfaction du cerveau avec des drogues osmotiques ; un drainage thérapeutique du fluide cérébrospinal (FCS) par le biais de cathéters intraventriculaires ; des tomographies assistées par ordinateur (CT-scans) ; une sédation ; une analgésie ; une relaxation musculaire ; une hyperventilation modérée ; une hypothermie modérée ; et un coma barbiturique.

22. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où l'animal a souffert de LME, et lequel anticorps ou fragment de celui-ci se liant à un antigène est pour une utilisation en conjonction avec un protocole sélectionné dans le groupe consistant en : une administration de stéroïdes ; une immobilisation de la colonne vertébrale ; une décompression chirurgicale ; une chirurgie pour stabiliser les vertèbres ; une chirurgie pour réaligner les vertèbres ; et une traction.

23. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon n'importe laquelle des revendications 1 à 23, où l'animal est un mammifère, de préférence où l'animal est un humain.

24. L'anticorps ou le fragment de celui-ci se liant à un antigène pour une utilisation selon la revendication 1, où le symptôme de LTC est présenté par des spasmes vasculaires cérébraux, une dépression cardiovasculaire, une hypotension, une hypoxémie, une hypercarbie, une hypoglycémie, une déficience neurologique post-traumatique, et une mort cellulaire neuronale ; et/ou où le symptôme de LME est présenté par une tuméfaction de la moelle épinière, un déficit dans la fonction motrice, ou un déficit dans la capacité de marche.

25. Une composition pour une utilisation dans la réduction ou la prévention d'un symptôme de lésion traumatique cérébrale (LTC) ou de lésion de la moelle épinière (LME) chez un animal ou pour favoriser la récupération après une LTC ou une LME chez un animal, laquelle composition comprend :

a) un premier agent sélectionné dans le groupe consistant en : un anticorps isolé ou un fragment de celui-ci se liant à un antigène qui se lie de façon sélective au facteur B, au facteur D, ou à la properdine et inhibe leur activité ; et
b) un deuxième agent pour une utilisation dans le traitement d'un symptôme de LTC ou de LME.

26. La composition pour une utilisation selon la revendication 25, où l'anticorps isolé ou le fragment de celui-ci se liant à un antigène se lie au facteur B au sein du troisième domaine de la séquence consensuelle répétée (SCR) et inhibe ou empêche la formation d'un complexe C3bBb.

27. La composition pour une utilisation selon la revendication 25 ou la revendication 26, où l'anticorps ou le fragment de celui-ci se liant à un antigène est : a) l'anticorps monoclonal 1379 (produit par ATCC Dépôt N° PTA-6230) ou un fragment de celui-ci se liant à un antigène ; ou b) un anticorps monoclonal ou un fragment de celui-ci se liant à un antigène qui se lie au même épitope sur le facteur B que mAb 1379, de préférence où l'anticorps ou le fragment de celui-ci se liant à un antigène est une version humanisée, ou chimérique de mAb1379 ou un fragment de celui-ci se liant à un antigène.

28. La composition pour une utilisation selon la revendication 25, où le deuxième agent est un composé pour traiter un symptôme de LTC sélectionné dans le groupe consistant en : une déficience physique, une déficience cognitive, et une déficience psychosociale-comportementale-émotionnelle.

29. La composition pour une utilisation selon la revendication 25, où le deuxième agent pour traiter une LTC est sélectionné dans le groupe consistant en : une drogue osmotique, un sédatif, un analgésique, un relaxant musculaire, et un barbiturique.

30. La composition pour une utilisation selon la revendication 25, où le deuxième agent pour traiter une LME est un

stéroïde.

31. La composition pour une utilisation selon la revendication 25, où le symptôme de LTC est une déficience physique, une déficience cognitive, une déficience psychosociale-comportementale-émotionnelle, ou une mort cellulaire neuronale, et/ou où le symptôme de LME est une tuméfaction de la moelle épinière.

FIG. 1

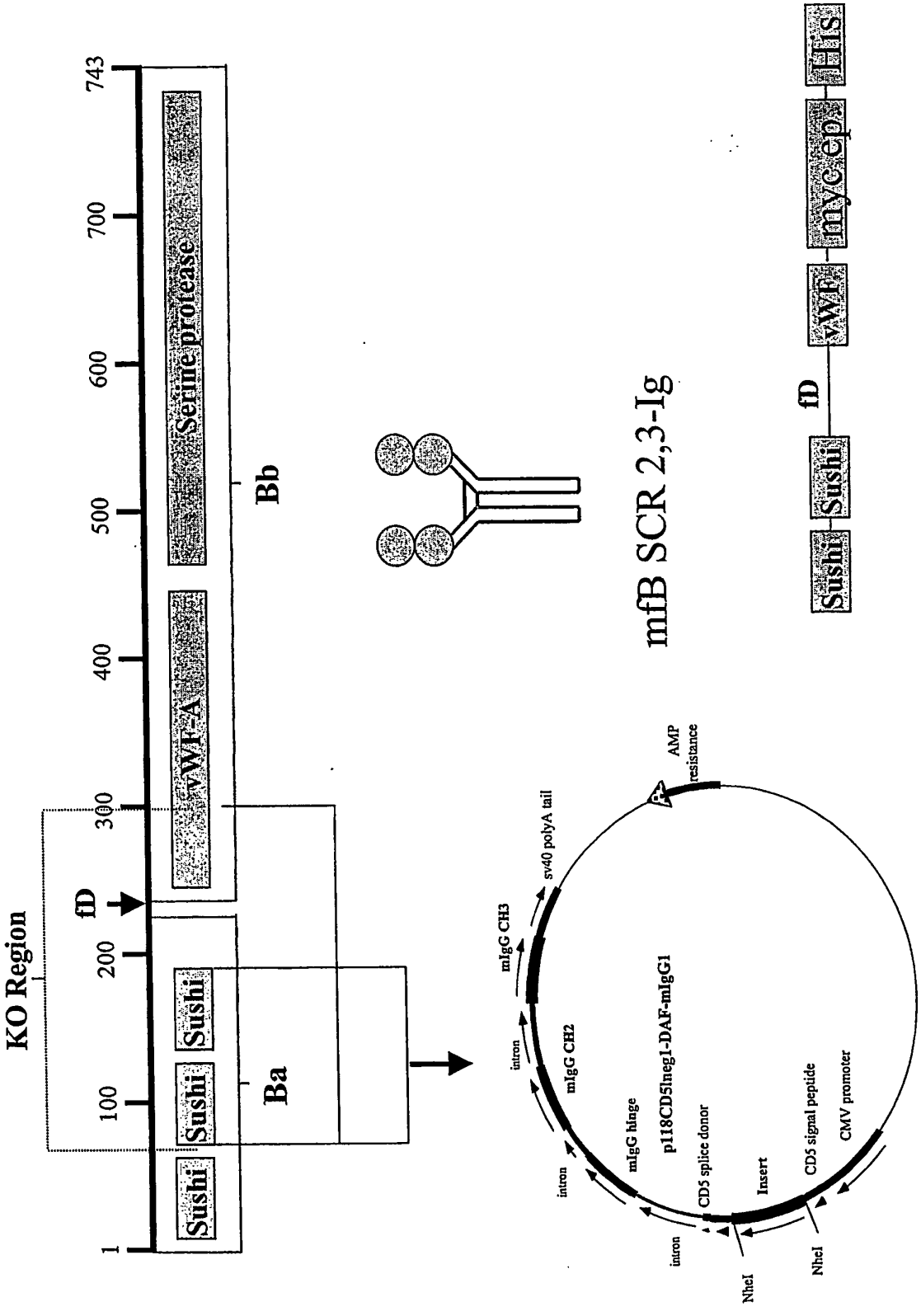

KO Region
fD
Sushi
Sushi
Sushi
vWF-A
Serine protease
Ba
Bb
1
100
200
300
400
500
600
700
743
mfB SCR 2,3-Ig
fD
Sushi
Sushi
vWF
myc ep.
HIS
mIgG CH3
sv40 polyA tail
AMP resistance
intron
mIgG CH2
intron
mIgG hinge
p118CD5ineg1-DAF-mIgG1
CD5 splice donor
intron
Insert
NheI
CD5 signal peptide
NheI
CMV promoter

FIG. 2A

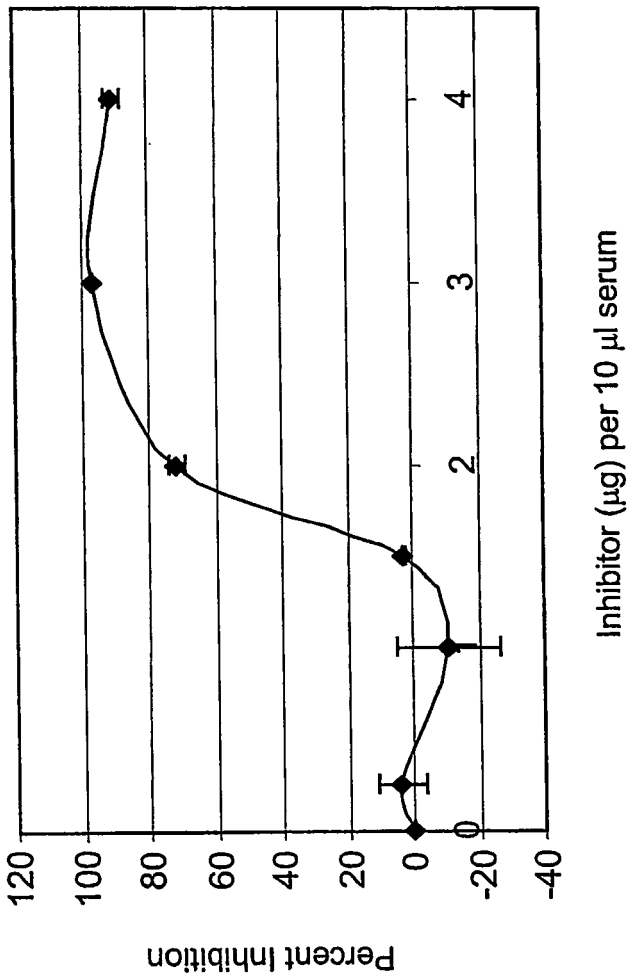

Percent Inhibition
120
100
80
60
40
20
0
-20
-40
0
2
3
4
Inhibitor (µg) per 10 µl serum

FIG. 2B

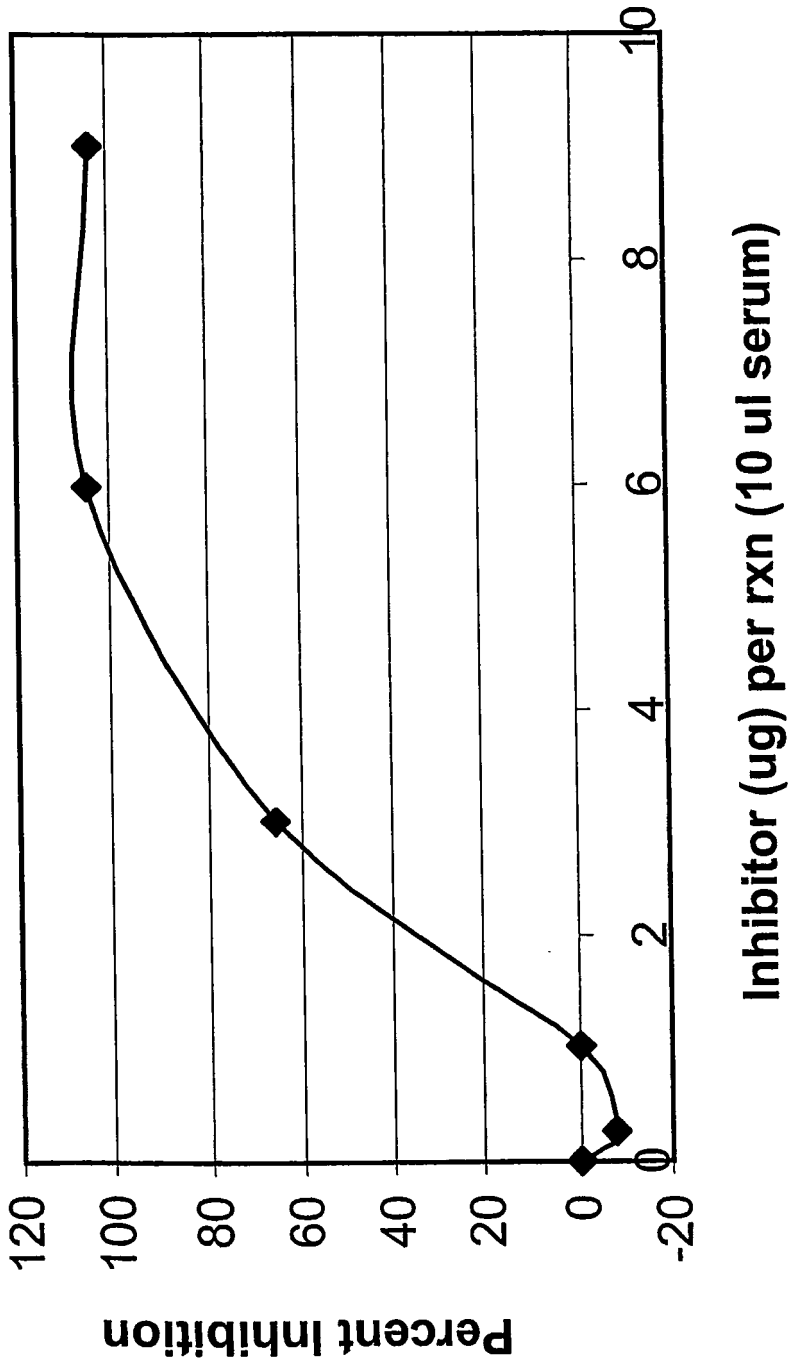

Percent Inhibition
120
100
80
60
40
20
0
-20
0
2
4
6
8
10
Inhibitor (ug) per rxn (10 ul serum)

FIG. 3

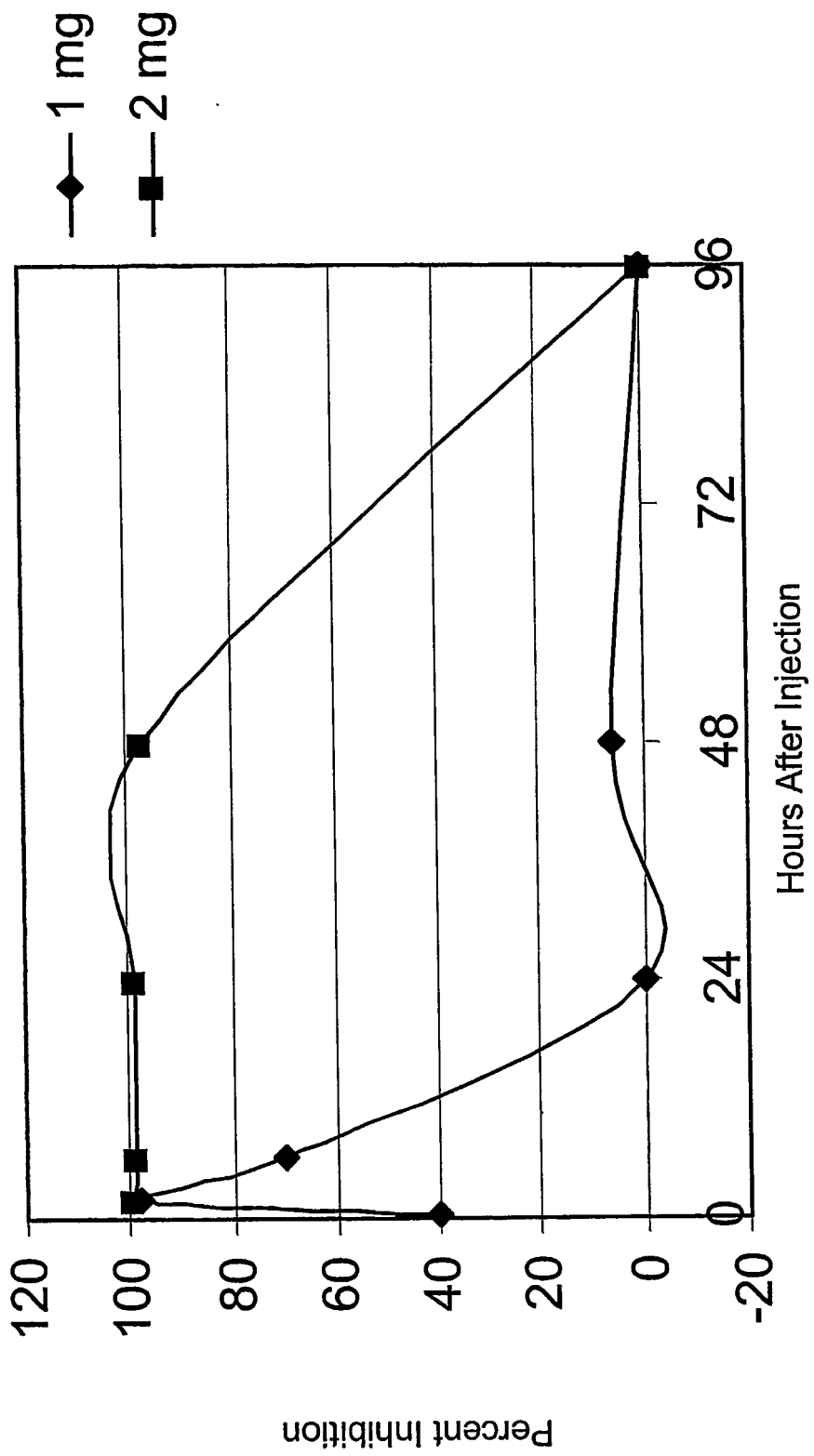

1 mg
2 mg
120
100
80
60
40
20
0
-20
Percent Inhibition
0
24
48
72
96
Hours After Injection

FIG. 4

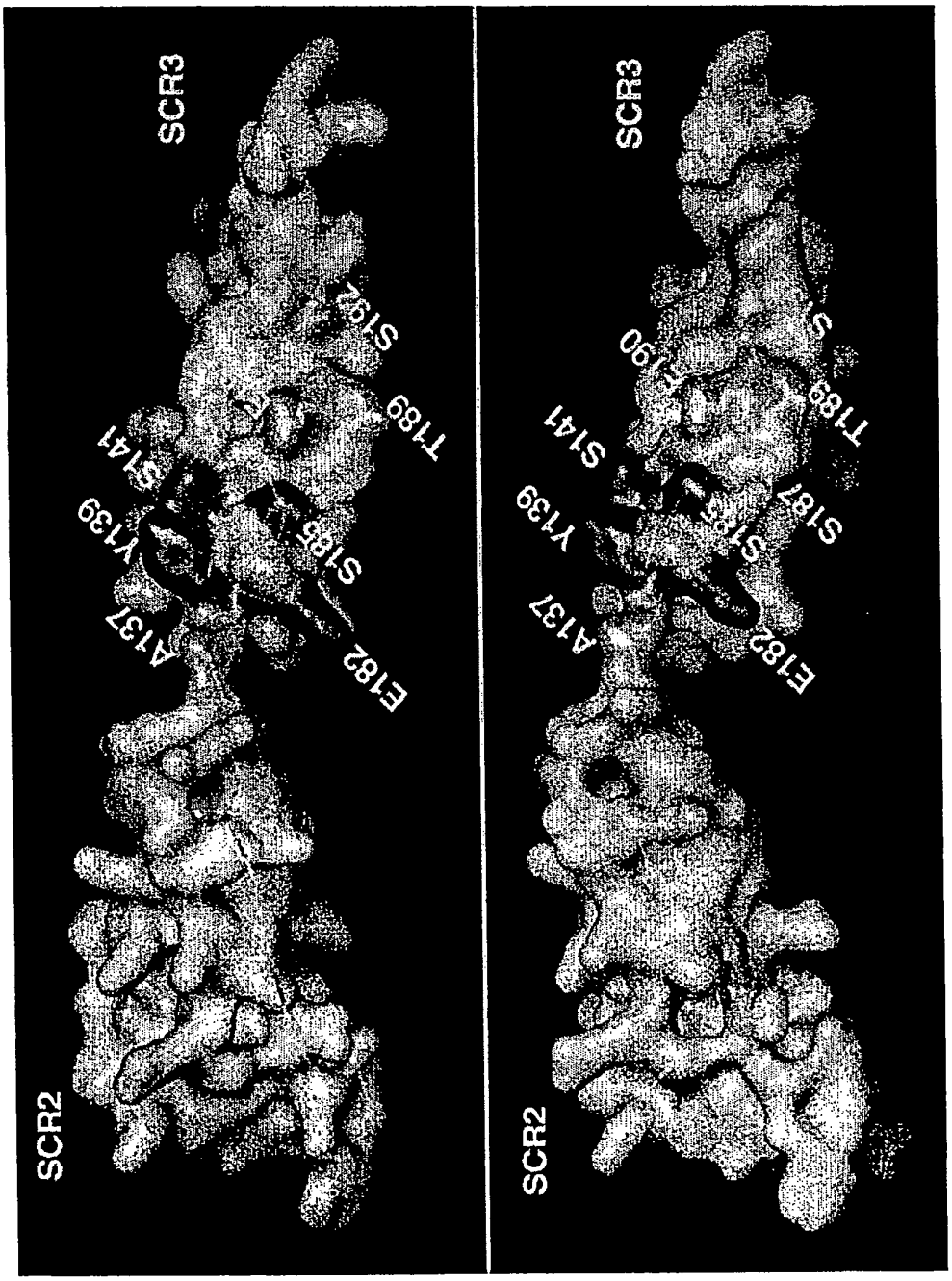
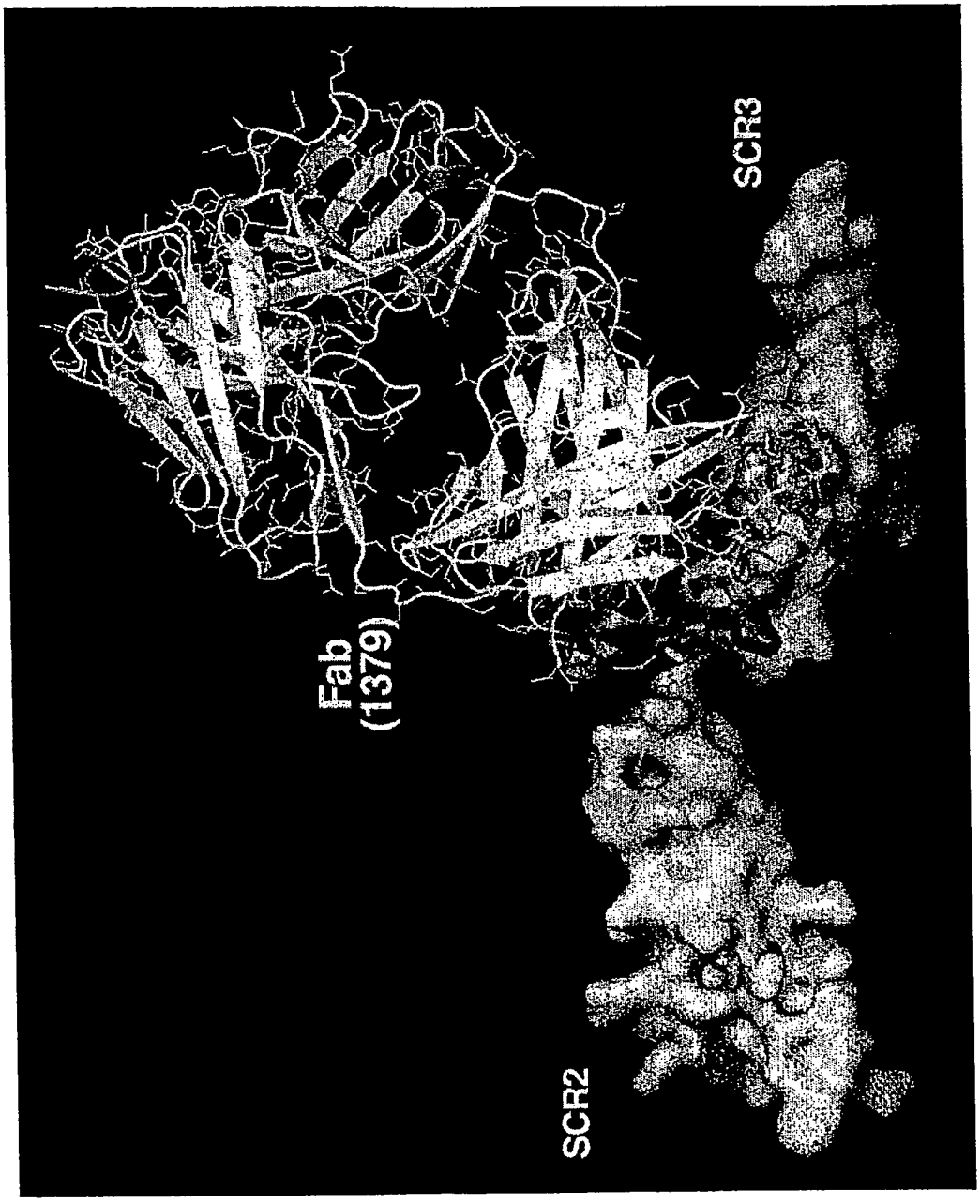

SCR3
SCR2
T189
S192
S141
V139
A137
S185
E182
SCR3
SCR2
T189
S187
S141
V139
A137
E182
S190

FIG. 5



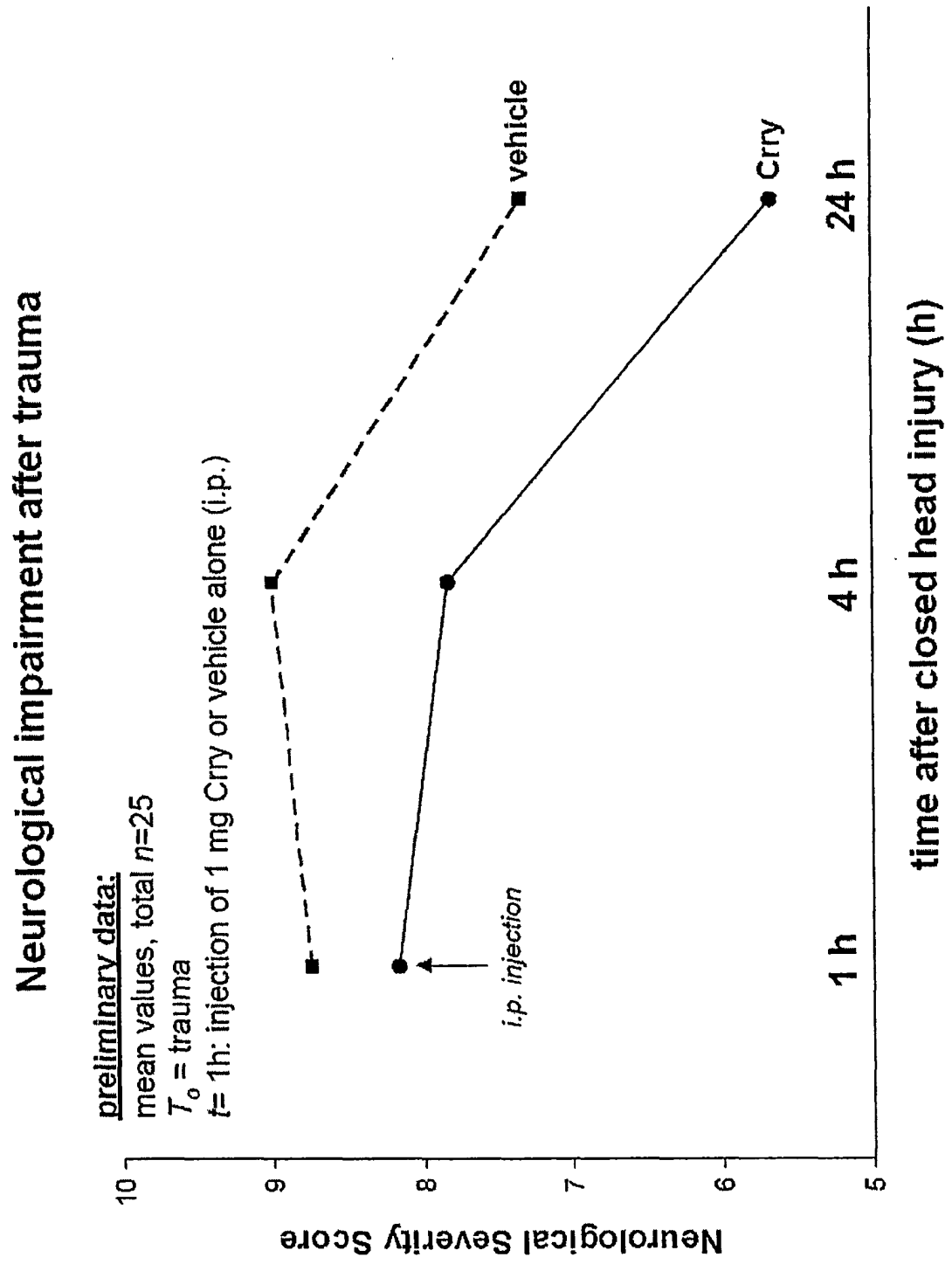

Neurological impairment after trauma
preliminary data:
mean values, total n=25
T_o = trauma
t= 1h: injection of 1 mg Crry or vehicle alone (i.p.)
Neurological Severity Score
10
9
8
7
6
5
vehicle
Crry
i.p. injection
1 h
4 h
24 h
time after closed head injury (h)

FIG. 6

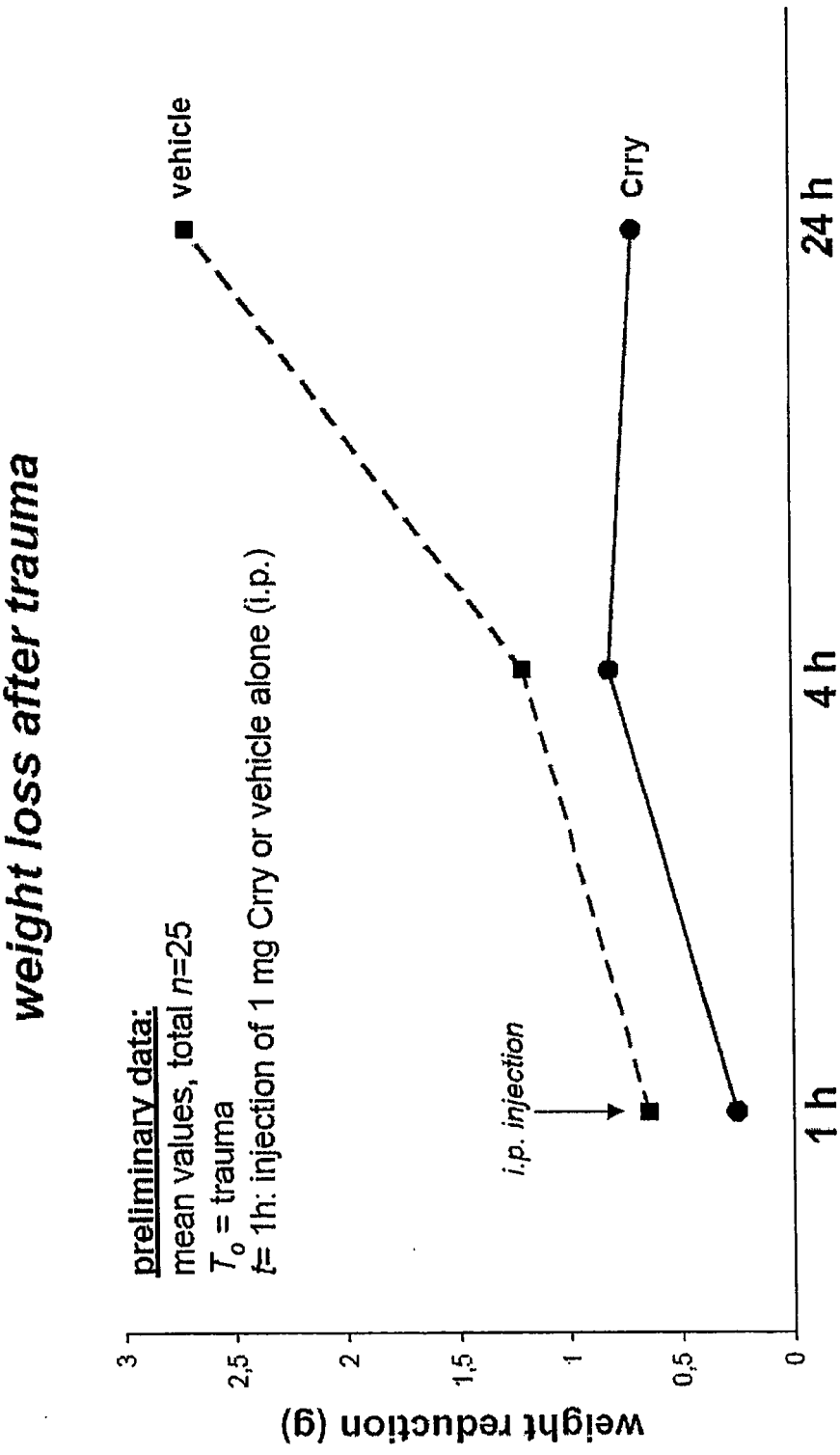

weight loss after trauma
preliminary data:
mean values, total n=25
T_o = trauma
t= 1h: injection of 1 mg Crry or vehicle alone (i.p.)
vehicle
Crry
i.p. injection
weight reduction (g)
3
2,5
2
1,5
1
0,5
0
1 h
4 h
24 h
time after closed head injury (h)

FIG. 7

FIG. 8

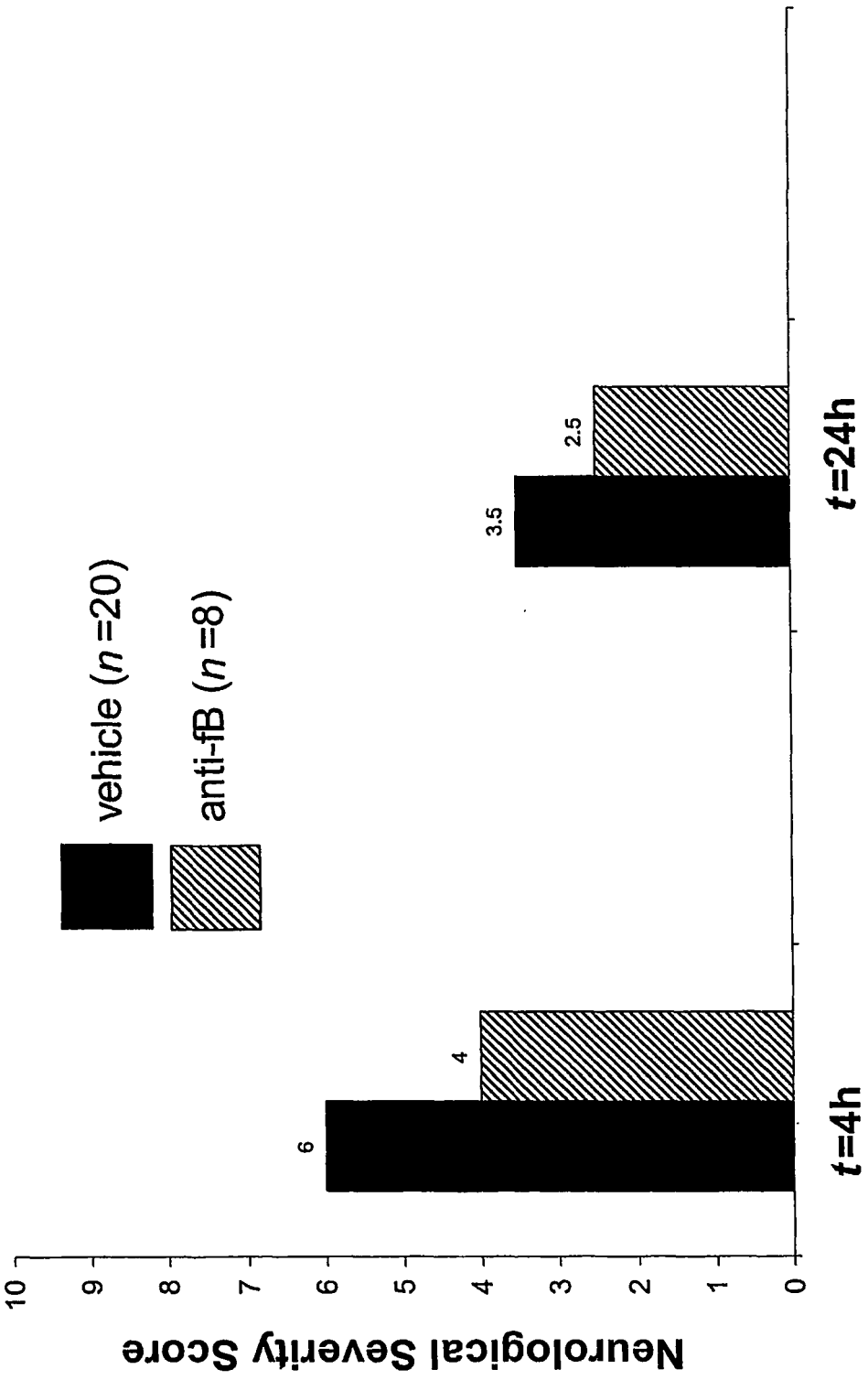

vehicle (n =20)
anti-fB (n =8)
Neurological Severity Score
0
1
2
3
4
5
6
7
8
9
10
6
4
3.5
2.5
t=4h
t=24h

FIG. 9

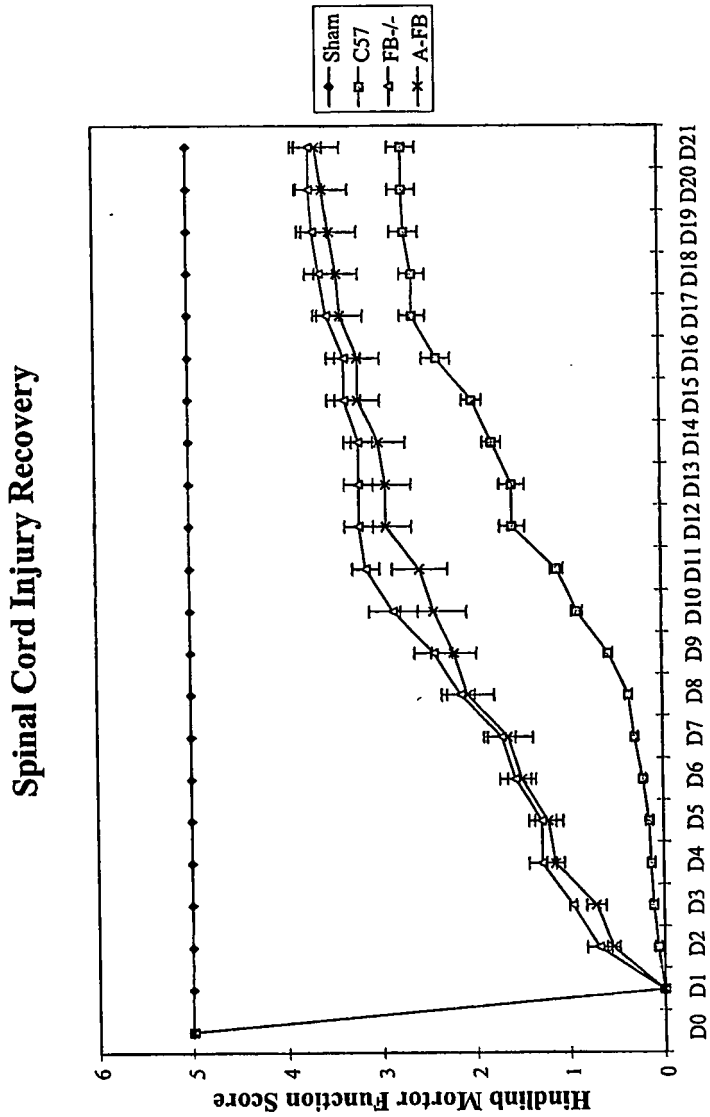

Spinal Cord Injury Recovery
Sham
C57
FB-/-
A-FB
Hindlib Motor Function Score
0
1
2
3
4
5
6
D0 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19 D20 D21
Time(Post-SCI)

FIG. 10

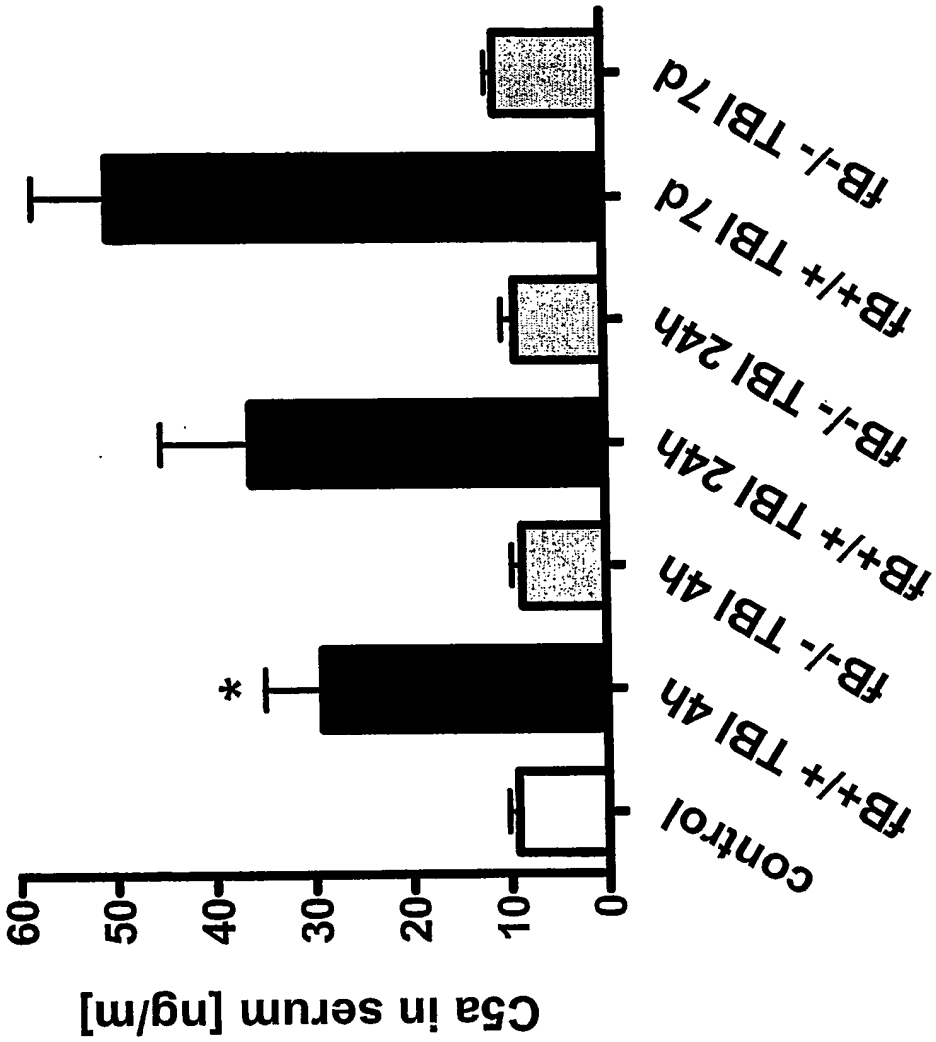

C5a in serum [ng/m]
0
10
20
30
40
50
60
*
Control
fB+/+ TBI 4h
fB-/- TBI 4h
fB+/+ TBI 24h
fB-/- TBI 24h
fB+/+ TBI 7d
fB-/- TBI 7d

FIG. 11

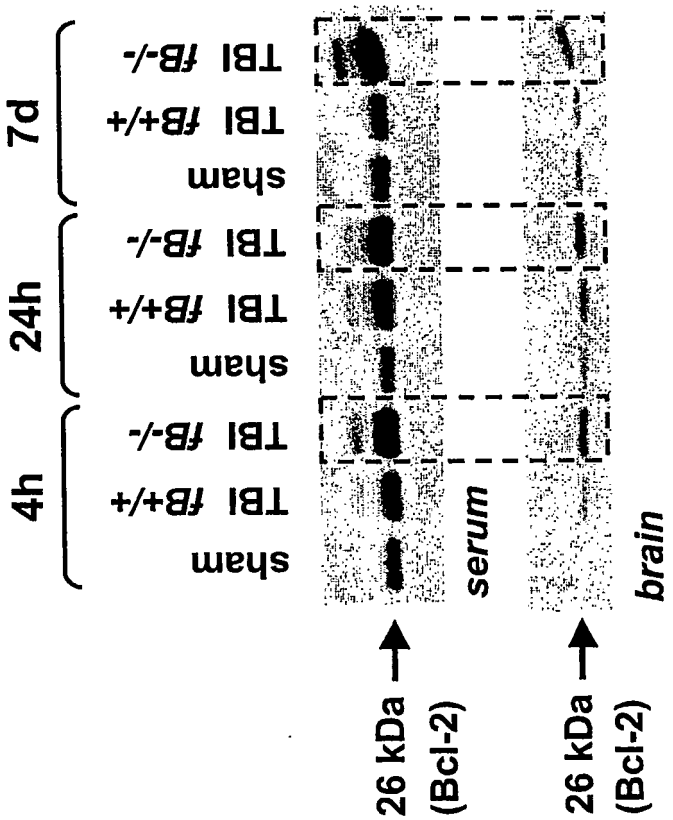

4h
24h
7d
sham
TBI fB+/+
TBI fB-/-
sham
TBI fB+/+
TBI fB-/-
sham
TBI fB+/+
TBI fB-/-
serum
brain
26 kDa
(Bcl-2)
26 kDa
(Bcl-2)

FIG. 12

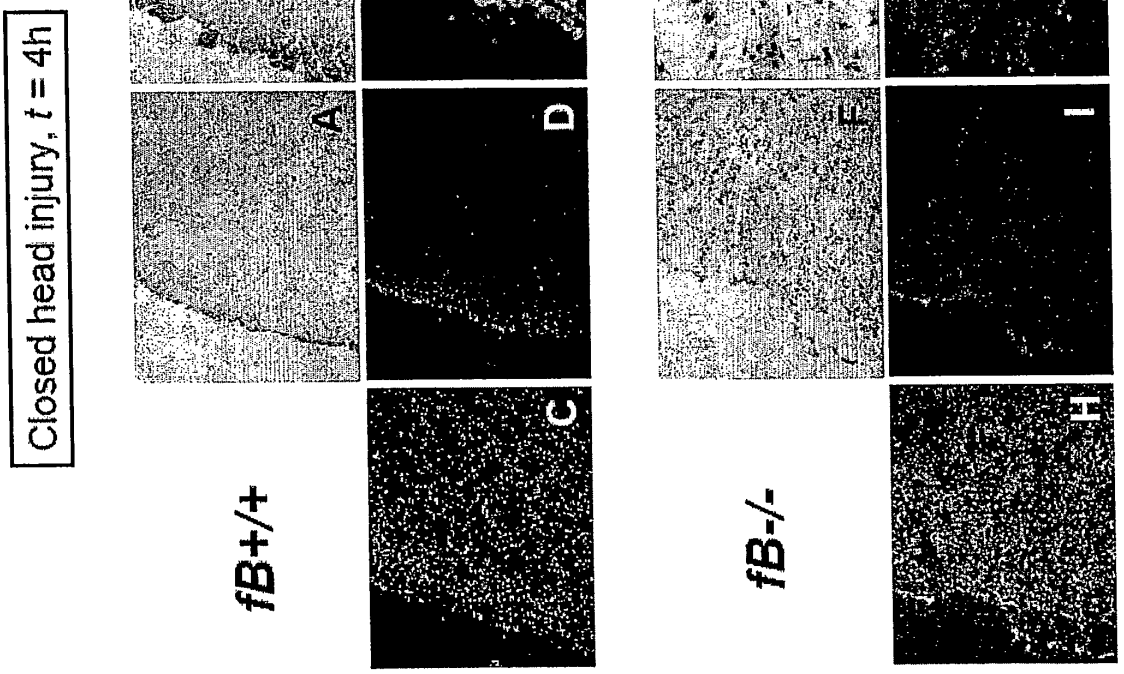
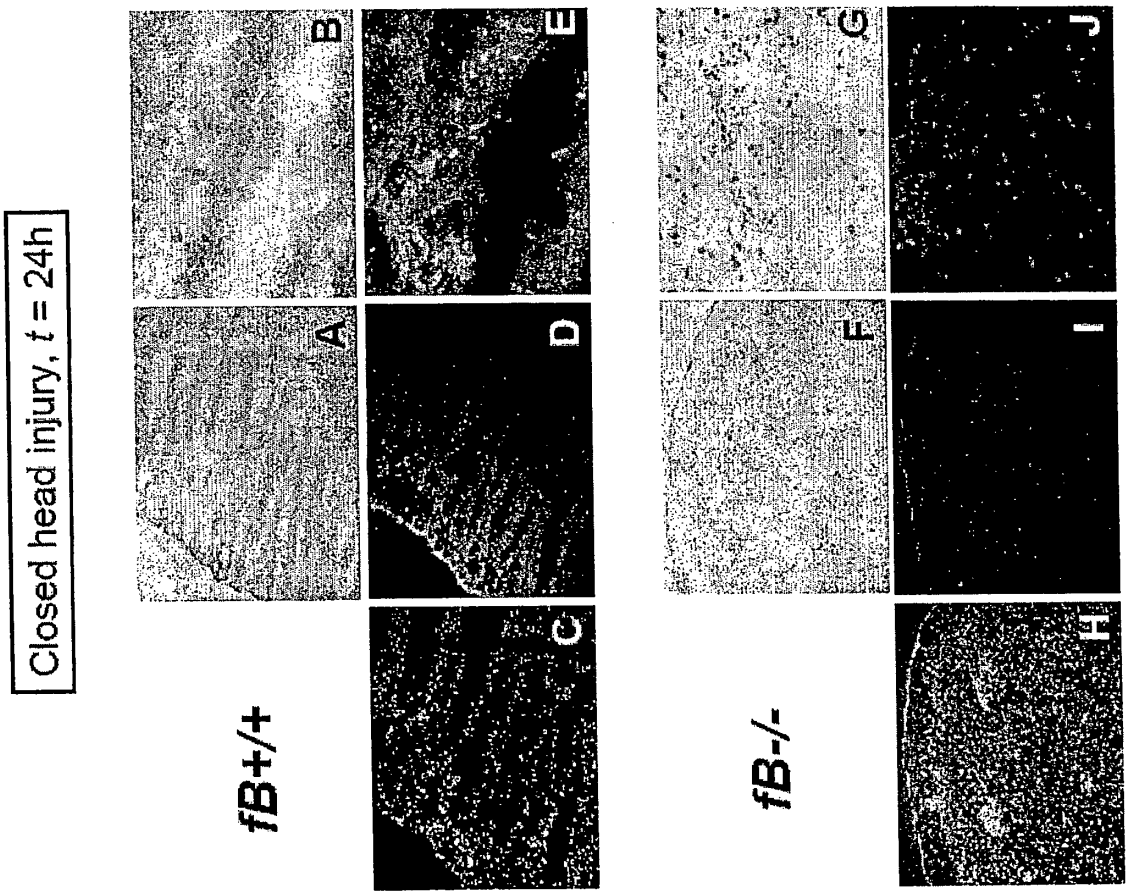


FIG. 13



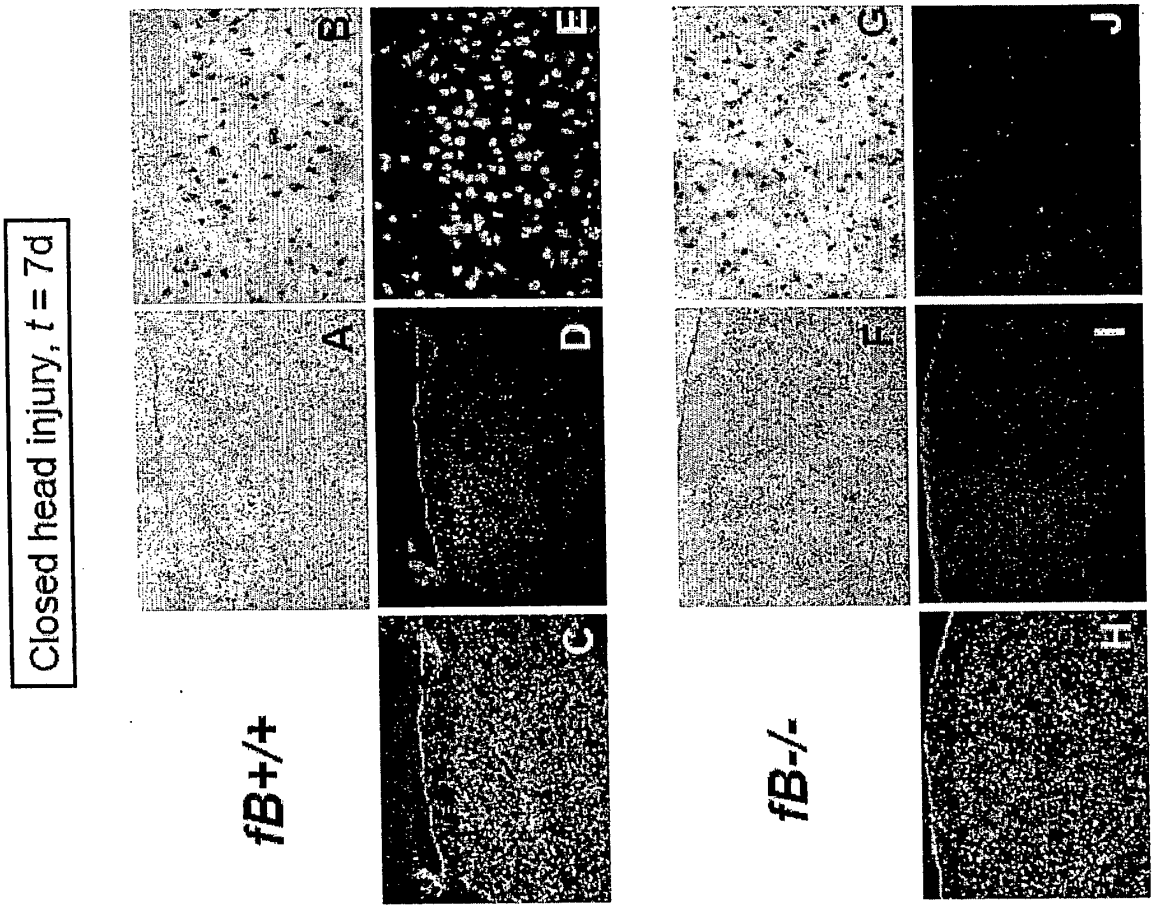


FIG. 14

REFERENCES CITED IN THE DESCRIPTION

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